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Determination of Sodium Chloroacetates in Cocoamide Propyl Betaine by Gas Chromatography: FID, ECD and MS.

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Abstract: This study regards a development of analytical method for determination of sodium monochloroacetate MCAS and sodium dichloroacetate DCAS incocoamidepropyl betaine CAPB by gas chromatography. CAPB is a feedstock for consumer products of the cosmetic and household segments and MCAS and DCAS are toxic, irritating and harmful impurities, so that, low contents are required (parts per million level) in process control of producers and regulatory affairs. To this end, the analytical method developed has: 1) appropriate performance parameters precision, accuracy and low quantification limit; 2) alternative detection modes by flame ionization FID and mass spectrometer MS, since the most conventional electron capture detector ECDrequires government control andqualified radiological protection from manufacturer due radioactive source ^{63}Ni; 3) efficient analyte derivation and separation by employ ethanol and liquid-liquid extraction, respectively. The validation process was applied to ensure a selective, robust, accurate and reproducible analytical determination for the developed methodologies.

Keywords: chloroacetate, cocoamide propyl betaine, gas chromatography, quality control.

I. INTRODUCTION

Cocamide Propyl Betaine (CAPB) is a technical grade chemical product categorized as an amphoteric surfactant because it consists a carboxylate group anionic and a quaternary nitrogen group cationic. CAPB is compatible with other surfactants and recognized as dirt remover, good foam stabilizer, stable over a wide pH range and with lower dermal and ocular irritability than the anionic surfactants, as reported in the technical literature of the manufacturers [1].

For these reasons, CAPB is applied in a wide range of formulations intended for personal and home care [2]- shampoos, conditioners, soaps, disinfectants and detergents - and as the surfactants thus applied are used in high volume and frequency in contact with the skin, this leads to the toxicological concerns.

According to illustrated by the reaction scheme of Fig. 1, CAPB is obtained by condensation between coconut fatty acidand dimethylaminopropylamine, initially forming the amidoamine intermediate, which in the next reacts with sodium monochloroacetate (MCAS) to form CAPB [3].

In the Table 1, CAPB constituents are presented, highlighting the chemical formulas and the categorization from the application point of view.

MCAS and DCAS, impurities present in CAPB, have a toxic effect when ingested or aspirated and an irritant effect in contact with the skin, as reportedin the literature and the safety data sheet from producers of Monochloroacetic Acid and Sodium Monochloroacetate [4], [5]. Therefore, strict controls of these impuritiesare mandatories and maximum limits of 20 mg / kg are required,according to the application of the CAPB, the regulatory aspects, the region and the industrial segment.

Among the available test methods in the CAPB matrix currently used by the industry, gas chromatographic with electron capture detection (GC-ECD) is widely used, but this kind of detector contains the radioactive source of $_{63}$Ni and the companies need licenses and controls from the Nuclear Energy National Commission (CNEN).

There is other inconvenient: ECD detector is less universal and their use is restricted to a few classes of analytes when compared to detectors by flame ionization (FID) and by mass spectrometry (MS). As a consequence, it is important to develop alternatives in GC to determine chloroacetates in amphoteric surfactants by the FID and MS detection modes, in view of the higher availabilityof these equipment in the chemical industry and more universal response to the different classes of chemical compounds [6].

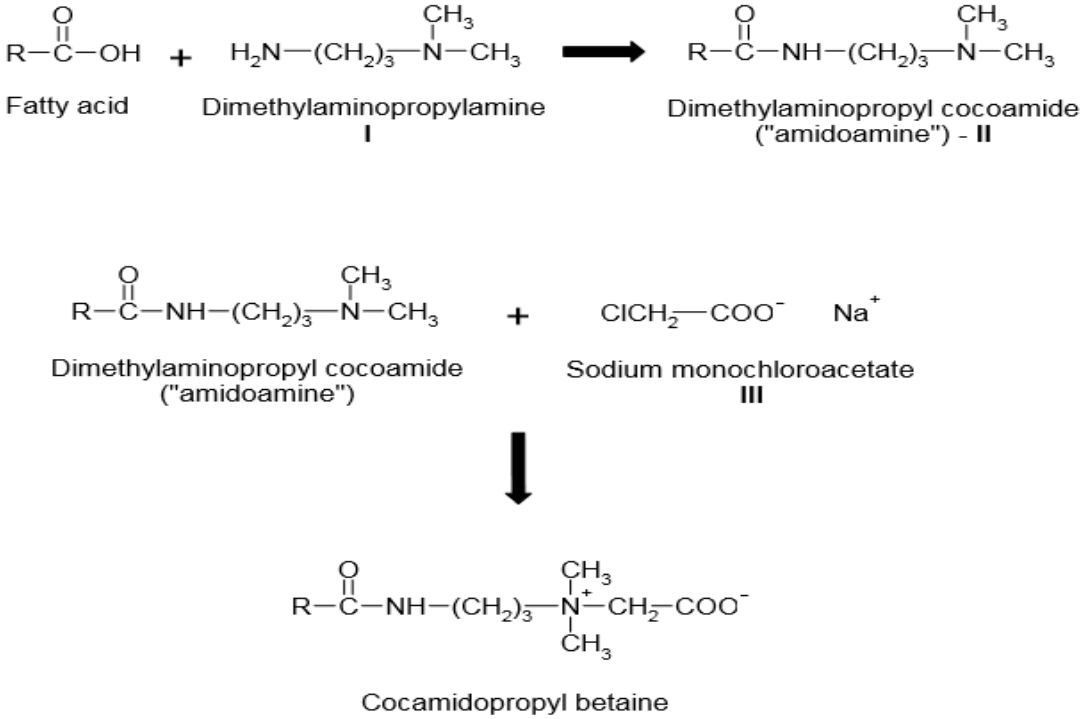
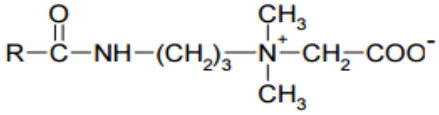
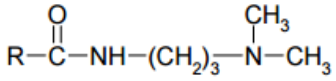
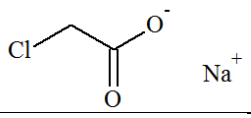
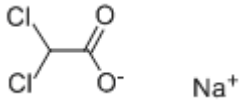
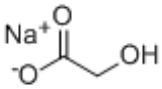


Figure1 Synthesysof CAPB from the feedstocks coconut fatty acid, dimethylaminopropylamine and MCAS.

Table 1 Constituents of Cocoamide Propyl Betaine (CAPB)

Substance	Structural Formula	Function
Cocoamidopropyl betaine (R = C8 to C18)		Active ingredient
Dimethylaminopropylcocoamide (amidoamine)		Residual intermediate
Sodium Chloride	Na+ Cl-	By-product
Sodium Monochloroacetate		Residual raw material
Sodium Dichloroacetate		Impurity present in raw material MCAS
Glicolato de sódio		By-product
Dimetilaminopropylamine		Residual raw material

The chemical species of interest in this study are MCAS and DCAS impurities, which need to be derived to esters and separated from the other constituents of the CAPB matrix to be quantified by gas chromatography. The methodology for determining the MCAS and DCAS contents in CAPB employed the esterification of the analytes of interest with ethanol in acid medium, extraction in hexane medium and quantitation by gas chromatography coupled to the FID, ECD and MS detectors using calibration curve, as detailed in the section II.

This paper describes the development, validation and comparison of analytical methodologies for determination of impurities MCAS and DCAS in CAPB matrix by gas chromatography with detection modes flame ionization (GC/FID), electron capture (GC/ECD) and mass spectrometry (GC/MS). Methods and its metrological requirements support the user to adopt the procedure that contemplates the availability of technology and the proper performance for the quality control regime [7], as noted in the section III.

II. METHODOLOGY

The determination of the MCAS and DCAS in CAPB consists of two steps:

• preparation of the sample for the injection in the chromatograph, whose purpose is to obtain volatile, thermally stable and partially separated substancesfrom the other constituents of the matrix [6];
• chromatographic determination, which is the separation of the species of interest present in the extract from the rest of the matrix and the subsequent quantitative measurements [8].

A. Sample Preparation

The preparation of the samples and standards containing the MCAS and DCAS is initiated by derivation of the analytes into more volatile compounds through esterification in acid medium with ethanol [9].The esterification consisted of the conversion of the carboxylic acids MCAS and DCAS to the corresponding ethyl esters, as demonstrated by equations (1) and (2), by displacement of the active hydrogen of the carboxylic acid by an alkyl group, so that the derivatives formed have lower polarity and greater thermal stability [10].

$$ClCH_2CO_2H + CH_3CH_2OH \longrightarrow ClCH_2CO_2CH_2CH_3 + H_2O \quad (1)$$

$$Cl_2CHCO_2H + CH_3CH_2OH \longrightarrow Cl_2CHCO_2CH_2CH_3 + H_2O \quad (2)$$

Note: other methods for esterification with methanol [11] and propanol [12] are also reported in the literature.

The liquid-liquid extraction combined with derivation enhances the recovery of MCAS and DCAS and its separation, detection and quantification by gas chromatography [10]. In order to complement the sample preparation, this study uses the extraction with the non-polar organic solvent n-hexane, taking advantage of the different solubilities of the formed esters and other constituents of the matrix [13].

The chemical materials used in the present study are:

• Cocoamido Propyl Betaine, CAS 61789-40-0, technical grade from brazilian manufacturer, content 30 %;
• Monochloroacetic Acid, CAS 79-11-8, analytical reagent from Fluka Sigma-Aldrich, content 100 %;
• Dichloroacetic Acid CAS 79-43-6, analytical reagent from Merck, content 99.3 %;
• Absolute Ethanol CAS 64-17-5, analytical reagent from Alphatec, content 99.7 %;
• Sulfuric Acid CAS 7664-93-9, analytical reagent from Alphatec, content 95 – 99 %;
• Mixture of Hexane Isomers CAS 110-54-3, HPLC grade reagent from EMD, minimum content 98.5 %;
• Sodium Chloride CAS 7647-14-5, analytical reagent from Merck, content 99.9 %.

The solutions used in the sample preparation step are:

• 10 % Sodium Chloride: weigh10 g of sodium chloride accurately 0.1 g for 100 mL of demineralized water;
• Stock solution of the analytical calibration curve: weigh 0.09 g of monochloroacetic acid and 0.09 g of dichloroacetic acid with a minimum precision of 0.001 g for 100 mL of demineralized water volumetrically;
• Standard solutions of the analytical calibration curve: take aliquots from stock solution and dilute with Absolute Ethanol P.A. in volumetric flask, as illustrated in the Table 2.

Table 2 Volume of aliquots and volumetric flasks for the preparation of the calibration curve

Standard	Volume of Aliquot, mL	Volumetric Flask, mL	Concentration, mg/kg
1	1.0	250.0	3
2	1.0	100.0	8
3	1.0	50.0	16
4	3.0	100.0	24
5	3.0	50.0	48

The standards of thecalibration curve and the samples under examination (CAPB)are prepared according to the steps described below:

• Add volumetrically to headspace vial 3 mL of standard or 1 mL of CAPB sample and 2 mL of Ethanol;
• Add approximatelly to each vial 0.2 mL Sulfuric Acid;
•Seal the vials and introduce into the oven set at 100 °C and during 20 minutes;
• Remove vials from the oven, stabilize at room temperature, add approximatelly 5 mL of 10% Sodium Chloride and add volumetrically 5 mL of Hexane;
•Introduce the magnetic stir bars, seal the vials and shake during 5 minutes;
•Wait for full phase separation and transfer the upper phase -Hexane medium - to the 2 mL vial.

In the next step, chromatographic injection of standards and samples is carried out.

B. Chromatographic Determination

GC determination uses the external standard method, which compares the analyte area in the CAPB sample with the areas obtained from calibration curve. Through linear regression the equations are established and concentration of the sample is determined from the area normalization procedure [14].

The equipment and accessories used in the present study are:

• Shimadzu GC2010 equipped with Electron Capture Detector (ECD) and Flame Ionization Detector (FID), data microprocessor and GC Solution program;
•Shimadzu GC2010 Plus coupled to the Shimadzu QP2010 SE mass spectrometry with electron ionization detector (MS), data microprocessor and GC/MS Solution program;
•OpenChrom: free and open source software for viewing and processing data in chromatography and spectrometry, from the native files of vendor's programs, such as, Agilent, Shimadzu, Thermo Fisher and Perkin Elmer;
•DB1 or DB5 capillary columns, length 30 m, internal diameter 0.32 mm and film thickness 0.25 μm;
• Oven from Perkin Elmer GC Autosystemadapted as stove for the esterification reaction, programmed to stabilize at 100°C for 20 minutes.

The chromatographic operating conditions are:

• GC/FID and GC/ECD: injector temperature 260°C, injection volume 1.0 μL, injection mode split 1:20, column furnace set with isotherm at 50°Cduring 2 minutes, heating at 10°C/min, isotherm at 120°C during 2 minutes, heating at 30°C/min and isotherm at 250°Cduring 1 minute, Helium or Hydrogenflow 1 mL/min, detector temperature 300°C, retention time 5.2 ± 0.2 min for ethyl monochloroacetate and 6.5 ± 0.3 min for ethyl dichloroacetate;
• GC/MS: injector temperature 275°C, injection volume 1.0 μL, injection mode split 1:10, column furnace set with isotherm at 50°Cduring 4 minutes, heating at 10°C/min, isotherm at 60°C during 1 minute, heating at 7°C/min, isotherm at 120°Cduring 1 minute, heating at 30°C/min and isotherm at 280°Cduring 1 minute Helium flow 1 mL/min, scanning in the SIM mode by fragments monitoring with m/z 49, 51, 79, 83 and 85, detector temperature 300°C, retention time 5.5 ± 0.2 min for ethyl monochloroacetate and 7.6 ± 0.2 min for ethyl dichloroacetate.

When we consider the conventional modes of GC detection, FID does not have the specificity provided by GC/MS [15], nor the GC/ECD sensitivity [16], however FID is the least complex, most universal and most widely used in industrial quality control, not requiring the radioactive source present in the ECD, nor a more sophisticated technology ofMS.

C. Validation of Method

In this study, the analytical determinations involve separation techniques for impurities with content < 0.1% using a calibration curve, therefore the validation parameters used are: selectivity, linearity, precision, accuracy, detection limit, quantitation limit, working range and robustness [17], [14].

In theTable 3is summarized the series of experiments carried out to validate the methodologies.

Table 3 Performance Parameters and Analytical Method Validation Strategy

Performance Parameter	Assay Validation
Selectivity	Calibration curve by set of 5 standard solutions with and without the presence of the matrix, 3 replicates
Linearity	Calibration curve by set of 5 standard solutions, 7 replicates
Precision	Dispersion of results in the CAPB matrix, 7 replicates
Accuracy	Recovery test by standard addition in 2 levels of concentration, 3 replicates
Detection and Quantitation Limits	Calibration curve by lower concentration strategy, 7 replicates
Robustness	Variations in optimized analytical conditions during development

D. Analytical Uncertaint

According to the International Metrology Vocabulary [19], the uncertainty of a measurement is a parameter associated with the result which characterizes the dispersion of values around the mean. In this paper, the sources of uncertainties was identified and quantified in order to estimate the influence on the final measurement and an analysis of all these uncertainties led to the estimation of the combined uncertainty [20].

The following steps were carried out to evaluate the measurement uncertainty [21]:

• Mathematical models were defined;
• Components of uncertainty were established;
• Probability distributions were estimated;
• Standard uncertainties were calculated;
• Combined and expanded uncertainties were obtained to express the results.

III. RESULTS AND DISCUSSION

This section describes the optimization, validation and comparison of the methodologies developed and the interpretations of the results obtained in each of these stages.

A. Optimization

The derivation step need to obtain the maximum conversion of chloroacetic acids to esters and was achieved by the use of anhydrous ethanol - less toxic than methanol whose additional carbon benefits GC/FID and GC/MS sensitivity - and by reaction in oven with electronic controllfor temperature in (100.0 ± 0.5) ºC.

The liquid-liquid extraction was standardized for the separation of MCAS and DCAS analytes from the other constituents of the CAPB matrix by magnetic stirring that forms a gentle vortex for 5 minutes and allows a complete emulsification of the aqueous and organic phases.

Chromatographic determination was intended to obtain response, resolution, symmetry and sensitivity for the MCAS and DCAS analytes in the detection modes MS, ECD and FID.

In the GC/MS, scan by selected ion monitoring (SIM) with restricted number of fragments instead of Full Scan mode, injection with flow division (split), oven and injector temperature setting temperature programming and injector temperature were setted up, to get gain in resolution, response, symmetry and presumably also in sensitivity, as shown in Fig. 2.

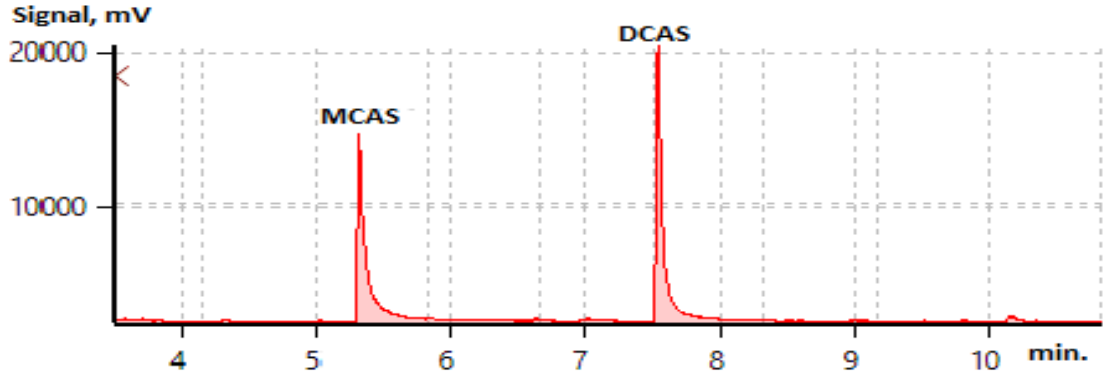


Figure 2GC/MS obtained by monitoring three specific mass/charge for MCAS 16.8 mg/kg at retention time 5.5 min and DCAS 18.4 mg/kg at 7.5 min.

The solutions of calibration curve by GC/MS were injected into the GC/ECD and produced adequate resolution and intense response for MCAS and DCAS at the retention times as indicated in the Fig. 3.

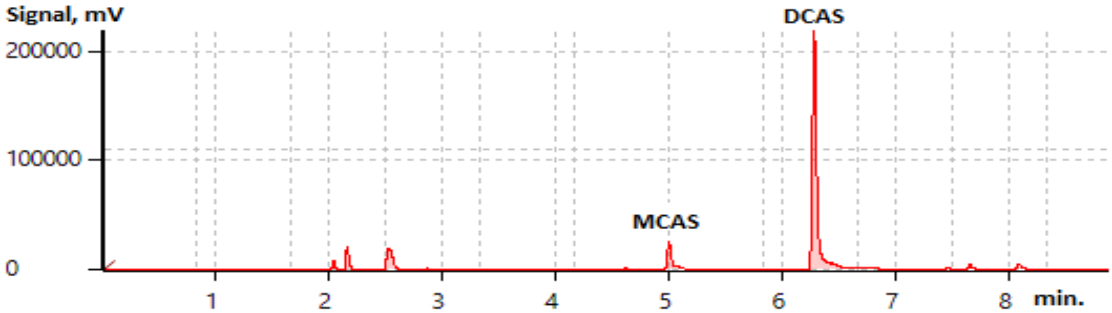


Figure 3 GC/ECD obtained for MCAS 16.8 mg/kg at retention time 5.0 min and DCAS 18.4 mg/kg at 6.3 min.

In addition, the result by GC/ECD signaled to adopt the same optimization criteria from MS mode to FID mode, regarding injector temperature, volume and injection mode, carrier gas flow, make up and type of column, obtaining the chromatogram ilustrated in the Fig. 4.

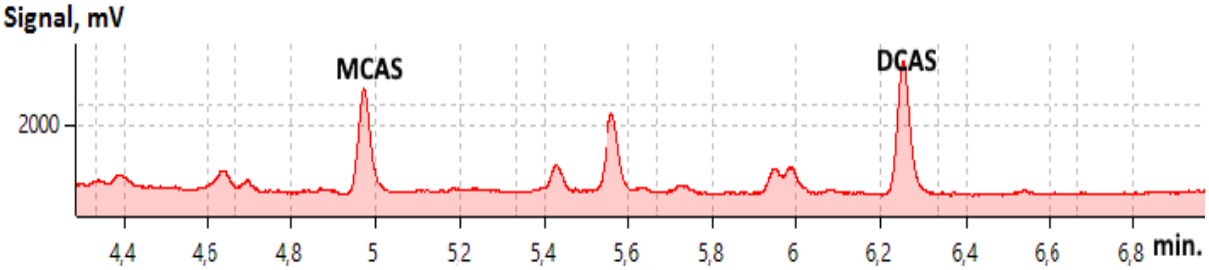


Figure 4 GC/FIDobtained for MCAS 16.8 mg/kg at retention time 5.0 min and DCAS 18.4 mg/kg at 6.3 min.

Identification and quantification of MCAS and DCAS analytesby three detection modes allowed the construction of calibration curves and the validation of the respective methodologies, whose performance parameters are reported at the following section.

B. Validation

Methodologies were evaluated based on some of the performance parameters [22], performing assays with standard solutions and CAPB matrix and by area normalization of chromatographic peaks.

Selectivity was qualitatively studied for three detection modes by peaks separation in the CAPB matrix, according to illustrate in the Fig. 5 for specific case of GC/FID chromatographic determination.

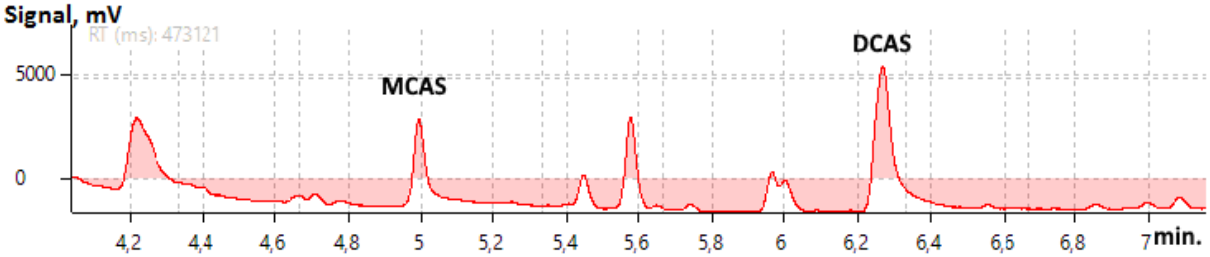


Figure 5 Example of selectivity by GC/FID showing peak separation of MCAS and DCAS at retention times 5.0 and 6.3 minutes, respectively, in the CAPB matrix.

Selectivity was also quantitatively studied by the Fisher-Snedocor distribution, which measures the ratio of two independent variances, calculated according to equation (3) and compared to a tabulated value:

$$F = \frac{s_1^2}{s_2^2} \quad (3)$$

where:
F is the Fisher-Snedocor distribution
S_1^2 and S_2^2 are variances of injections, with and without matrix assays, respectively;

The *F*-test was applied to standards of the analytes with and without the presence of the matrix, obtaining the results presented in the Tab. 4.

Table 4 Selectivity by F-test application to GC/ECD, GC/FID and GC/MS, considering *F*-critical value = 4.28.

Standard Solution, mg/kg	***F*-statisticfor MCAS**			***F*-statisticfor DCAS**		
-	ECD	FID	MS	ECD	FID	MS
4	1.59	3.01	1.33	3.97	3.33	2.77
9	1.38	2.61	1.39	1.20	1.95	6.46
18	3.97	1.48	6.01	5.94	2.27	5.59
26	6.58	5.13	3.89	2.28	1.55	11.39
52	1.47	7.44	1.42	2.81	8.50	3.77

By three detection modes there are some results that *F*-statistic >*F*-critical value. It was not definitive to consider the null hypothesis, that is, the matrix effect is present.

Although the methodologies are qualitatively selective, in a quality control systematic it is recommended to construct calibration curves in the presence of the CAPB matrix. Since the matrix effect is present in the three detection modes, a possible solution is to employ a polar column with higher polarity [23].

Linearity was determined from the linear regression between analyte content and area under the peak chromatographic, expressed by classic equation (4) and *R*-squared, according to reported byTable 5.

$$y = ax + b \quad (4)$$

where:
y is the measured response (area under the chromatographic peak);
x is the concentration of the analyte;
a is the angular coefficient (slope of the curve);
b is the linear coefficient (intersection of the curve with the y-axis).

Table 5 Parameters of linear regression to MCAS and DCAS by GC/ECD, GC/FID and GC/MS

Methodology	**Linear Equation**	***R*-squared**
MCAS by GC/ECD	Y = 20490x + 223273	0.992
MCAS by GC/FID	Y = 1451x + 1921	0.987
MCAS by GC/MS	Y = 7875x - 33397	0.997
DCAS by GC/ECD	Y = 126399x + 2420902	0.990
DCAS by GC/FID	Y = 982x + 8126	0.985
DCAS by GC/MS	Y = 9676x - 23285	0.998

Working range was detailed in addition to linearity for each analyte, by analyzing the standard deviation of the residuals by Student's *t*-test, calculated according to equation (5), whose values are presented in the Table 6.

$$t = \frac{res}{\frac{s_r}{\sqrt{n}}} \quad (5)$$

where:
t is the *t*-statistic;
resis the residue, difference between the measured value and the mean value;
$s_r$is the estimated standard deviation form residues;
nis the sample size expressed by replicates number.

Table 6*t*-test for residuals of linear regression considering *t*-critical value = 2.776

Standard Solution, mg/kg	***t*-statistic for MCAS**			***t*-statistic for DCAS**		
-	ECD	FID	MS	ECD	FID	MS
4	0.323	1.868	2.701	0.427	1.054	3.006
9	2.768	1.241	3.509	2.382	0.914	1.565
18	2.110	2.113	0.596	1.166	0.317	2.764
26	2.402	2.736	0.017	3.213	3.912	0.685
52	1.421	1.739	0.196	1.569	1.627	0.638

The three methodologies have linear response in the work range studied for MCAS and DCAS without presence of matrix, because they produced *R*-squared> 0.98 [24] with consistency of the linear regression to the experimental points evidenced by *t*-statistic <*t*-critical value.

. **Precision** as a dispersion of results was expressed by repetitiveness limit, according to equations (6) and (7).

$$s = \sqrt{\frac{\sum(x_i - x_m)^2}{n - 1}} \quad (6)$$

where:
s is the estimated standard value

x_i is the individual value of a measurement;
x_m is the arithmetic mean of a small number of measurements;
n represents the number of measurements.

$$r = t \cdot \sqrt{2} \cdot s \quad (7)$$

where:
ris therepetitiveness limit;
t is the Student's *t*-test for n-1 degrees of freedom and 95% confidence;
sis the estimated standard deviation calculated from equation (6).

The results presented in the Table7define the maximum differences of area allowed between replicates for the reported concentration level. In a quality control routine, replicates with differences greater than those reported in the Table 7indicate that the methodology is not precise and the root cause must be determined.

Table 7 Repetitiveness limit (r) for peak area of MCAS and DCAS without the presence of CAPB matrix

Standard Solution, mg/kg	**r calculated to MCAS**			**r calculated to DCAS**		
-	ECD	FID	MS	ECD	FID	MS
4	70128	1147	7448	251076	11021	647
9	76057	9449	2204	613161	21036	5032
18	520060	14484	16666	681592	9499	7279
26	789624	49523	43134	2440034	44041	9566
52	1989918	130374	136734	3864361	24254	359532

Repetitiveness between the techniques was not compared, since they have different levels of response: the ECD detection mode is more sensitive thanMS and FID modes, thus, the GC/ECD has larger differences between the replicate areas, without the same impact in the concentration.

Accuracy was tested by fortifying the CAPB matrix with the MCAS and DCAS at two concentration levels with triplicate determinations and measurement of recovered percentage and at TABLE 8 are presented relative standard deviation (RSD) and the recovery percentage.

Table 8 Percentage recovered of MCAS and DCAS in fortified CAPB samples

Detection Mode	**Concentration, mg/kg**		**RSD, %**		**Recovery, %**	
-	MCAS	DCAS	MCAS	DCAS	MCAS	DCAS
ECD	14.5	24.3	24	17	62	76
	39.7	45.0	17	19	85	102
FID	21.3	25.5	33	18	103	71
	40.2	46.2	19	21	113	86
MS	14.0	24.2	38	20	94	75
	39.2	44.9	12	14	92	95

Regarding impurities, RSD should not exceed 20 % close to quantitation limit and 15% in the rest of working range [24] and analogous studies that also involved low analyte concentrations predict a minimum recovery rate of 90% [9], [12], [15], [25], [26].

The recovery rates of 62% to 113% for MCAS and 71% to 102% for DCAS, affected by deviations of 12 to 38 %, point out the need to improve the methodology in order to avoid risks at analytes quantitation, even within the range of 70 to 120% acceptable for concentrations below 0.01% [14].

Limit ofDetection (LD) and **Limit of Quantitation** (LQ) were evaluated based on the analytical curve method [27] and calculated according to equations (8) and (9).

$$LD = t \cdot s \quad (8)$$

where:
LD is the limit of detection expressed as the analyte concentration;
t is the Student's *t*-test for n-1 degrees of freedom and 95% confidence;
sis the standard deviation from (6)for seven replicates of lower concentration of calibration curve.

.

$$LQ = x \,.\, 5 \,.\, s \tag{9}$$

where:
LQ is the limit of quantitation expressed as the analyte concentration;
x is the mean of the response for seven replicates of lower concentration of calibration curve;
sis the standard deviation from (6) for seven replicates of lower concentration of calibration curve.

In the TABLE 9 are presented LD and LQ for the three methodologies under study.

Table 9LD and LQ for MCAS and DCAS by GC/ECD, GC/FID and GC/MS

LD for MCAS, mg/kg			**LD for DCAS, mg/kg**		
GC/ECD	GC/FID	GC/MS	GC/ECD	GC/FID	GC/MS
1.0	1.1	0.5	0.7	2.2	0.5
LQ for MCAS (mg/kg)			**LQ for DCAS (mg/kg)**		
GC/ECD	GC/FID	GC/MS	GC/ECD	GC/FID	GC/MS
7.8	5.7	5.8	2.8	9.4	3.7

When we consider maximum 20 mg/kg for these analytesin some of the applications of CAPB in cosmetics and detergents, all LQ's at the TABLE 9 are lower than half of this specified limit and it is expected that methodologies are appropriate for quality control.

Robustness was not evaluated quantitatively since the methodologies are affected by deviations in the order of 20% and recovery rates of 70%, which jeopardize the judgment on which small changesaffect the performance of the method.

A qualitative evaluation of the results was carried out by experiments with different temperatures and time within the oven at esterification step, with the agitation mode for the extraction step and in the variation of the temperature programming of injector and column for the chromatography step, ensuring the relevance of controlling these conditions for the performance of the method.

Regarding the **uncertainty of results**, equations (10), (11) and (12) represent the mathematical models defined for solutions preparation and chromatographic determination.

$$C = \frac{1000 \,.\, m \,.\, P}{V} \tag{10}$$

where:
C is MCAA and DCAA concentration inmg/kg, at stock solution preparation;
mis MCAA and DCAA mass in mg, at weighing;
Pis the centesimal purity of MCAA and DCAA standards, dimensionless;
V is solution volume in L.

$$C_2 = \frac{C_1 \,.\, V_1}{V_2} \tag{11}$$

where:
C_2 is the concentration of diluted standard solution, in the dilutions of the stock solution;
C_1 is the concentration of stock solution;
V_1 is the aliquot of stock solution in L;
V_2 is the volumetric flask of standard solution in L.

$$[\text{MCAS}]\text{or}\,[\text{DCAS}] = \frac{(A - \text{Int}) \,.\, F}{\text{Slp}} \tag{12}$$

where:
[MCAS] or [DCAS] are the concentration of the analytesas calculated from the calibration curve;
A is the peak area at chromatographic run;
Int is thelinear coefficient, intercept of the calibration curve;
Slp is theangular coefficient, slope of the calibration curve.

The probability distributions for the chloroacetic acid purities, balance uncertainty, volume variation with temperature are rectangular and the divisor is square-root of three. The probability distribution of the lab

glassware uncertainty is triangular and the divisor is square-root of six. The probability distributions of solution volume, area peak repeatability, standard deviation of recovery that affects the intercept and slope of calibration curve are normal, the divisor is square root of replicates number.

The standard uncertainty is calculated from the standard deviations of the manufacturer, repetition or validation affected by the probability distribution divisors; the combined standard uncertainty is calculated by root sum of squares of each standard uncertainty (EURACHEM, 2002) and the expanded uncertainty is computed multiplying by coverage factor K equal to two.

The results for the expanded uncertainties are reported in the comparison of methodologies at TABLE10 in addition to the means and standard deviations.

The contributions of standard uncertainties to the combined uncertainty are quoted as illustrated by FIG. 6 and FIG. 7, exemplifying the preparation of DCAA standard solutions and the DCAS quantitation in CAPB by GC/ECD.

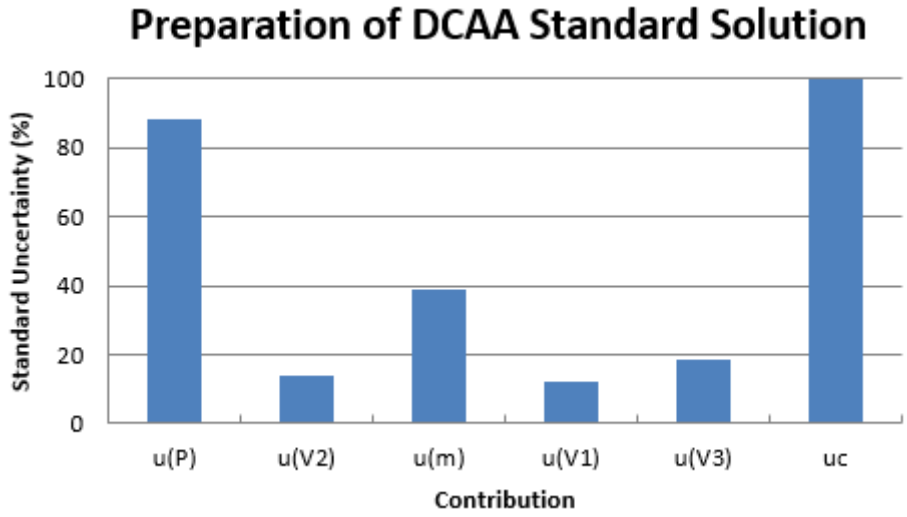


Figure 6 Standard uncertainties to prepare DCAA standard solutions: standard purityu(P), volume of the flask and pipetterepeatability u(V2), balance calibration u(m), volumetric flask calibration u(V1) and temperature influence on the volume change u(V3) for the combined uncertainty uc.

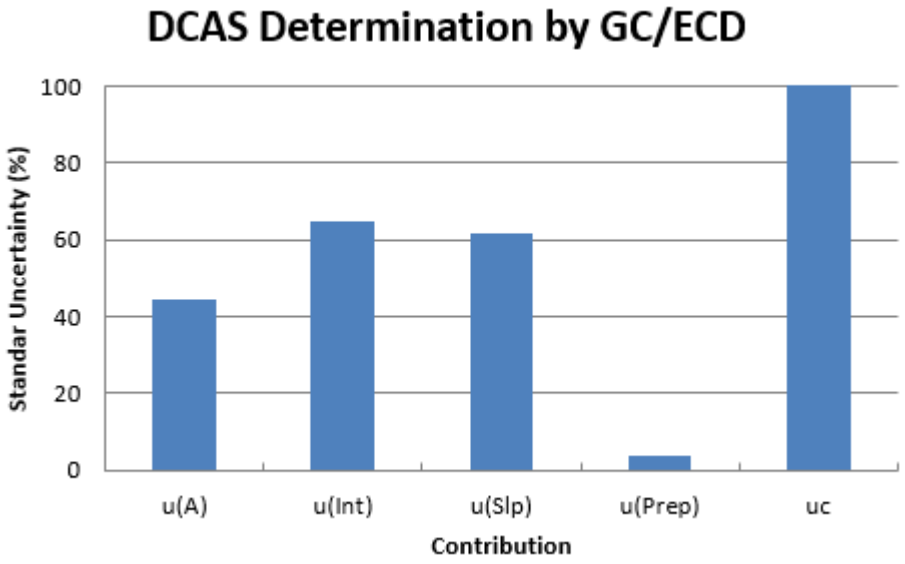


Figure 7 Standard uncertainties to determine DCAS by GC/ECD: peak areau(A), recovery assay deviation expressed at interception and inclination u(Int) and u(Inc) and standard solutions preparation u(Prep) for the combined uncertainty uc.

C. Method Comparison by Determination in a Commercial Sample

The measurement of equivalence between determinations in CAPB by GC/ECD, GC/FID and GC/MS also was compared by means for unpaired testing that produce results independently. The *t*-test was parameterized for independent samples and the results obtained are demonstratedin the TABLE 10.

Table 10 Comparisonfor unpaired testing to MCAS and DCAS determinations in CAPB sample

	GC/ECD vs GC/FID				**GC/ECD vs GC/MS**			
	MCAS		DCAS		MCAS		DCAS	
Mean Value	7.7	9.5	14.5	19.2	7.7	5.4	14.5	14.0
Standard Deviation	3.2	4.4	3.4	7.3	3.2	1.0	3.4	4.9
Variance	10.1	19.5	11.6	52.6	10.1	1.0	11.6	23.6
F2/F1	1.92	-	4.54	-	0.10	-	2.04	-
Degrees of Freedom	1.45	2.78	1.66	7.52	1.45	0.15	1.66	3.37
df-effective	5.5	6	6.9	7	0.016	1	6.5	7
t-critical	2.45	-	2.37	-	12.71	-	2.37	-
SD	2.055	-	3.029	-	1.264	-	2.243	-
t-effective	0.87	-	1.53	-	1.83	-	0.24	-

The performance methods are similar, since the comparisons express*t*-effective lower than *t*-critical.

The high standard deviations contribute to the similarity between the methodologies, since theaffects the meanand produce wide regions of coincidence between the sets of results, benefiting the comparison criteria, reducing the degrees of freedom and increasing*t*-critical.

The unpaired trials also aimed to obtain relative standard deviations below 20% and the results of mean and standard deviation calculated for seven replicates accompanied of expanded uncertainty obtained by EURACHEM simulated methodare reported at TAB. 11.

Table 11 Mean value, standard deviation and expanded uncertainty for MCAS and DCAS in CAPB sample

AnalyteandDetectionMode	**Mean Value, mg/kg**	**Standard Deviation, mg/kg**	**Expanded Uncertainty, mg/kg**
MCAS by ECD	7.7	3.2	4.8
MCAS by FID	9.5	4.4	2.5
MCAS by MS	5.4	1.0	1.1
DCAS by ECD	14.5	3.4	5.1
DCAS by FID	19.2	7.2	5.0
DCAS by MS	14.0	4.8	1.8

Relative standard deviation and expanded uncertainty more than 20% of the mean show the need to refine the methodologies.

In general, to improve studied methodologies in precision and accuracy, the following actions are recommended: improve time and temperature controls during esterification, use time-controlled magnetic stirring in extraction and have reproducible conditions for injection, heating and detection.

Specifically, performing calibration curves based on the surfactant matrix at the time of analysis, the increase in the number of replicates, the availableness of automatic injection and internal standardization are efficient strategies for gains in precision and accuracy.

IV. CONCLUSION

The procedures idealized in this paper for analytes derivatization with ethanol and subsequent extraction of the matrix with hexane allow the chromatographic determination using FID, ECD or MS detection.

The GC/ECD technique was improved and the development of the GC/FID and GC/MS methods provides alternatives for MCAS and DCAS determination in CAPB and enables the industrial quality control to decide the appropriate technique according to the resources available at laboratory.

The metrological requirements studied in the validation do not allow to highlight any method in relation to the others, demonstrating that the advantages and deficiencies are similar for GC/ECD, GC/FID and GC/MS and confirming the importance of sample preparation. In addition, validation indicates the need to improve the performance of the three techniques in selectivity, precision and accuracy.

The methodologies are linear in the studied range of 4 to 50 mg / kg. Determination factors greater than 0.99 and low residue deviations confirm the fit of the linear regression to the experimental points.

The obtained results in a commercial sample were similar, although the high measurement uncertainties increased the universe of probable results for the three techniques.

The experimental results for FID, ECD and MS detection modes presented equivalent performances. The FID presents the advantages of more universal application, lower cost, less complex proficiency and exempts the use of radioactive source in its operation.

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