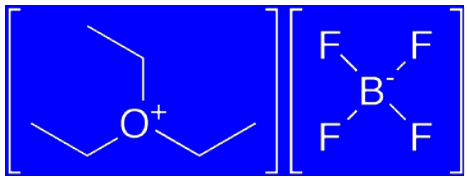
Optimization and analytical applications of chemical vapour generation of anionic species by aqueous phase alkylation with triethyloxonium salts

AIM OF THE WORK

alkylation of SCN^-, CN^-, S^{2-} anionic species in aqueous phase and HS/GC-MS analysis
Optimization of the reaction conditions pH, reaction time and Headspace condition such as temperature and salting-out effect.
Application of the method to the analysis of biological samples.
Triethyloxonium salts are able to give volatile alkyl derivate of some anionic species



The two competitive reactions of reagent are:

$$X^-_{(aq)} + R_3O^+_{(aq)} \rightarrow RX_{(g)} + R_2O \qquad \text{(derivatization reaction)}$$

$$R_3O^+ + H_2O \rightarrow R_2O + ROH + H^+ \qquad \text{(hydrolysis)}$$

In the table are reported the hydrolisys of different trialkyloxonium reagent:

Oxonium salt	***Hydrolisys time [min]***
$(Me_3O)^+BF_4^-$	8
$(Et_3O)^+BF_4^-$	80
$(n\text{-}Pr)_3O^+BF_4^-$	120

Some consideration of the main reactions of derivatization procedure can be postulated

Analytical process

$R_3O^+_{(aq)} + X^-_{(aq)} \rightarrow RX_{(aq)} + R_2O$ derivatization reaction

$RX_{(aq)} \rightleftharpoons RX_{(g)}$ phase transfer of analyte derivate

Competitive process

$R_3O^+ + H_2O \rightarrow ROH + R_2O + H^+$ reagent hydrolysis

$X^-_{(aq)} + H^+_{(aq)} \rightleftharpoons HX_{(aq)}$ protonation of analytical substrate

$HX_{(aq)} \rightleftharpoons HX_{(g)}$ phase transfer of protonated analyte

$RX + R_3O^+ \rightarrow R_2X^+ + R_2O$ alkylation of analytical derivate

Interfering reactions (matrix interferences)

$X^- + M^{n+} \rightleftharpoons MX^{(n-1)+}$ metal complex formation

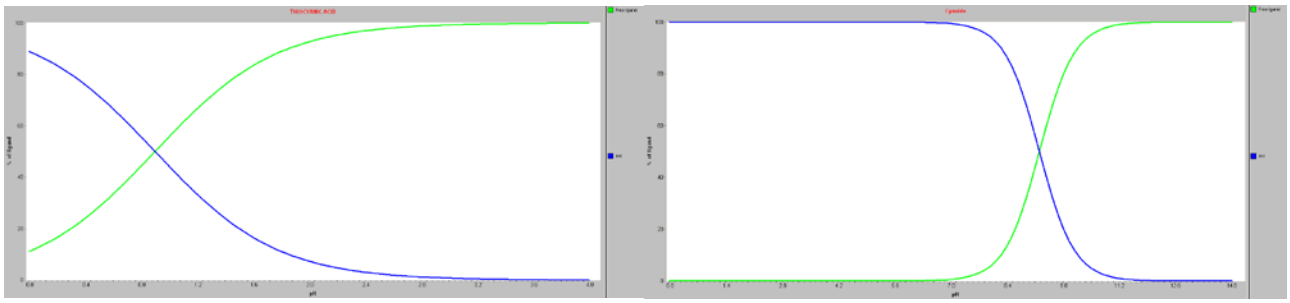
$R_3O^+ + Y^- \rightarrow RY + R_2O$ alkylation of anionic species other than X^-

$R_3O^+ + L \rightarrow RL^+ + R_2O$ alkylation of ligand/donor species

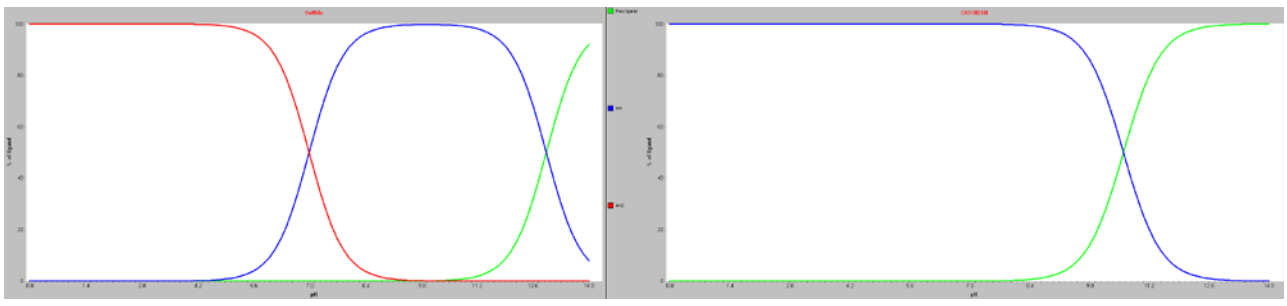
Several parameters can play an important role in this kind of reaction system and in this work they have been systematically investigated for the optimization of the analytical methods.

pH

The sulfide and cyanide are the coniugate base of very weak acid and they undergo easily to protonation and the resulting acid (H_2S and HCN)are volatile and easily escape from solution. Otherwhise the thiocyanate is coniguated base of relative strong acid.
In the picture below are reported the distribution of anionic species in function of pH:



For the sulfide is also reported the distribution species in function of pH of the ethylsulfide the first alkylation product of sulfide



The main responsibility of pH decrease is the reagent hydrolysis

$$R_3O^+ + H_2O \rightarrow ROH + R_2O + H^+$$

Central Composite design with ***two factors (k)*** and ***five levels***

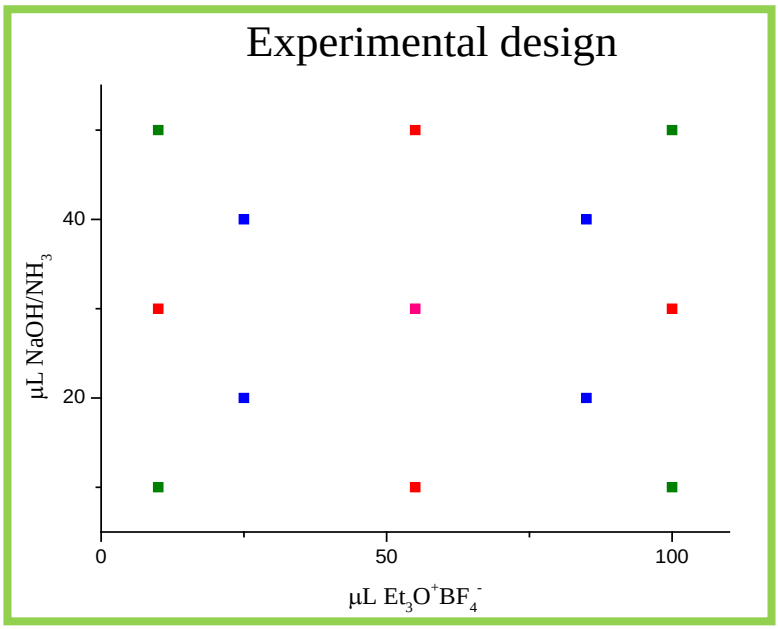
$$N = 2k^2 + 2k + 1$$

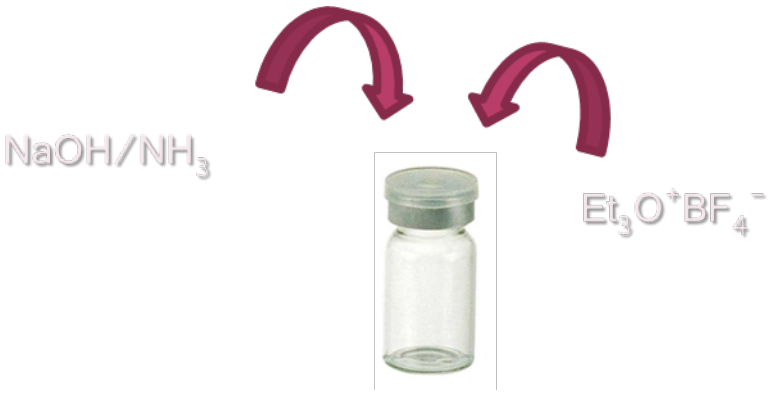
[NaOH] 0.02-0.1 M

[NH3] 0.02-0.1 M

[Et3O+BF4-] 0.01-0.1 M

100 µg/mL all of the anions



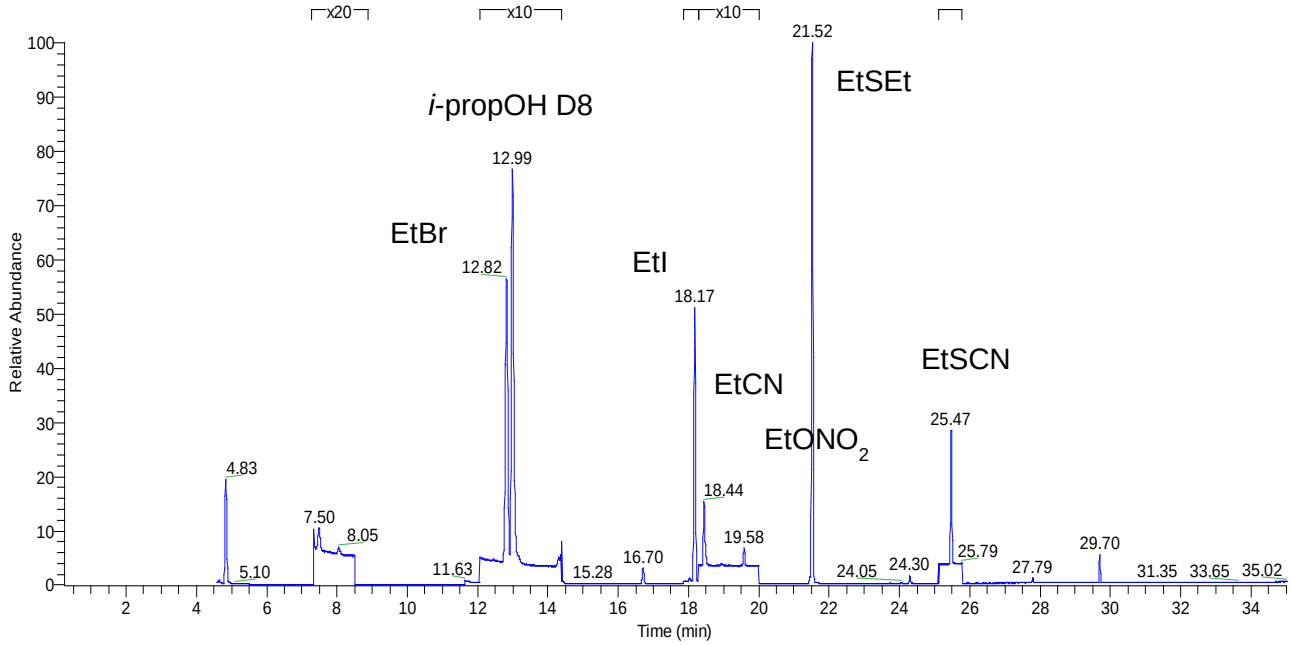


Chromatographic conditions

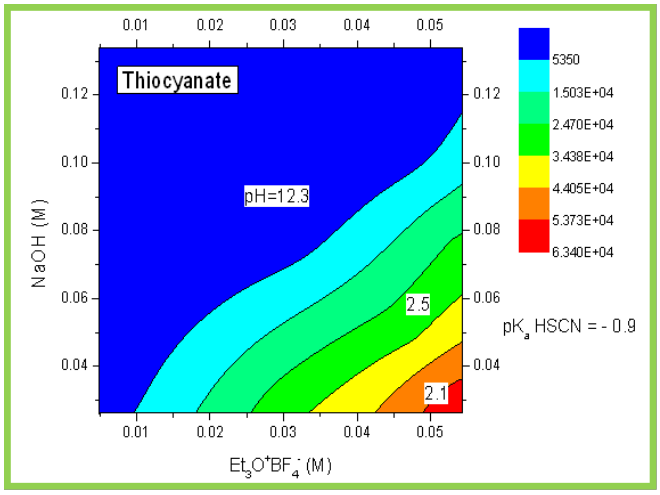
- Headspace Gas Chromatography/Mass Spectrometry (HS/GC-MS), equipped with an autosampler and an incubator 60 min at 60°C
- capillary column DB-624, 6% cyanopropyl-phenil 94% dimethylpolisiloxane (60m x 0.250mm x 1.40µm)
- Injection volume : 1000 µL
- carrier gas: helium (99.9995%)
- The transfer line, mass spectrometer and quadrupole temperature were respectively 260°C, 250°C and 150°C.

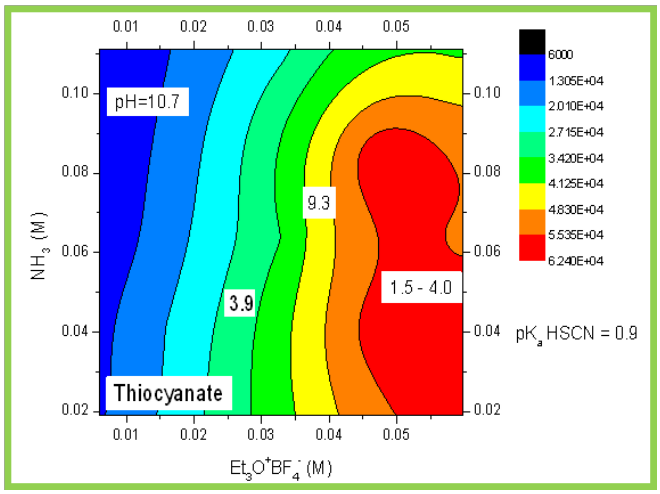
INTERNAL STANDARD

i-propyl alchol D8 (99.8%)

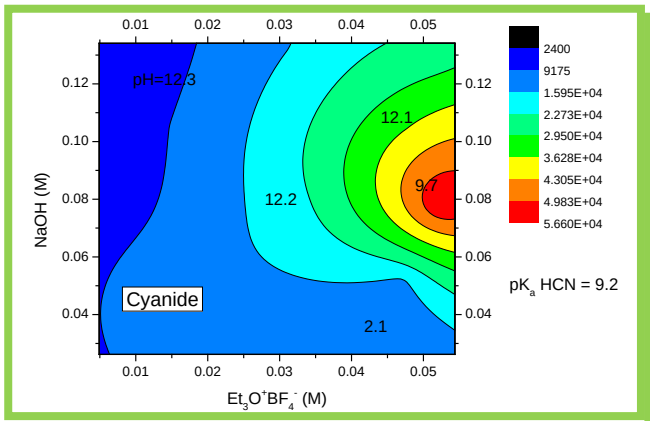


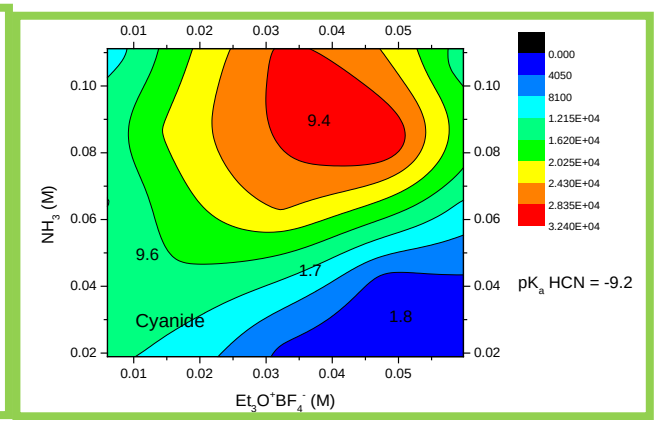
THIOCYANATE RESPONSE SURFACES



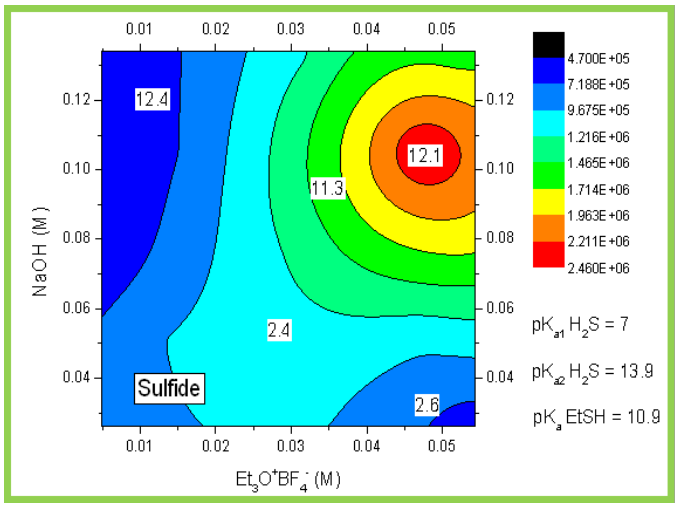


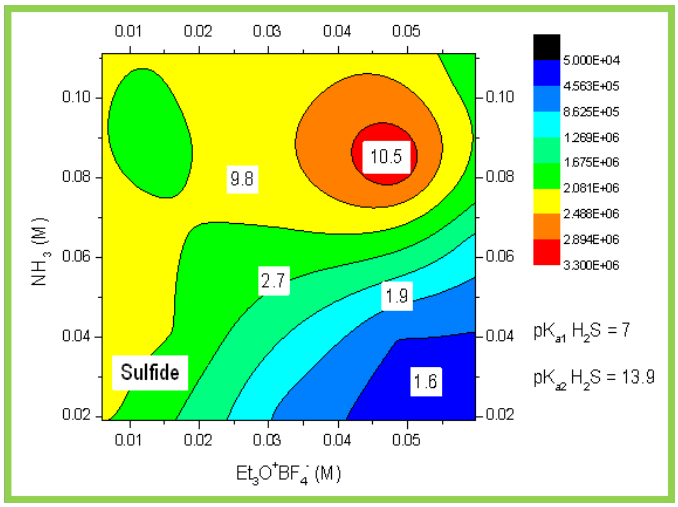
CYANIDE RESPONSE SURFACES





SULFIDE RESPONSE SURFACES





Temperature and salt addition were studied by Central Composite design with two factors (k) and five levels

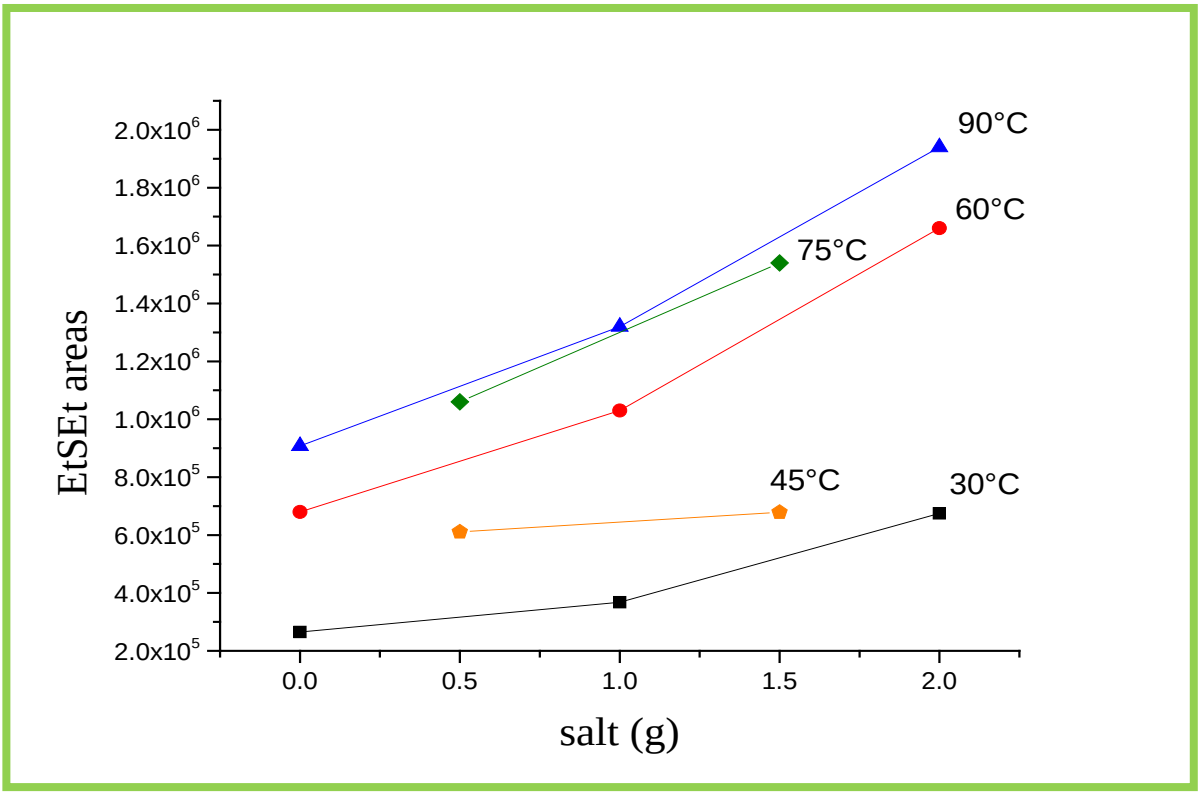
$$N = 2k2 + 2k + 1$$

WORKING SOLUTION: 3 µg/L of EtSCN, EtCN and EtSEt

SALT: 0-2 g Na2SO4·10H2O
TEMPERATURE: 30 – 90°C

Thirteen points of the experimental design

$g\ Na_2SO_4 \cdot 10H_2O$	$T\ [°C]$
0	30
1	30
2	30
0.5	45
1.5	45
0	60
1	60
2	60
0.5	75
1.5	75
0	90
1	90
2	90



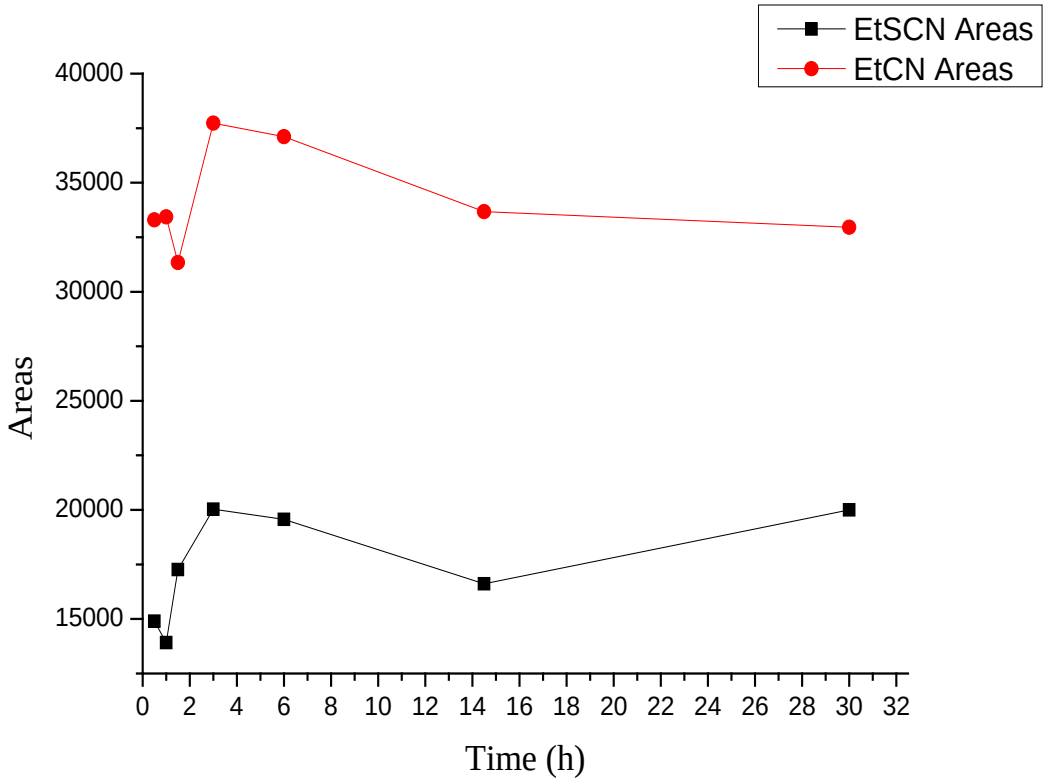
EXPERIMENTAL RESULTS

- *Effect of reaction time*

Time from the addition of the ethylating agent and the HS/ GC-MS

$$X^- + R_3O^+ \rightarrow RX + R_2O$$

30', 60', 90', 180', 360', 14h30' , more than 24h



The time elapsed from reagents addition and analysis for reaction reach a plateau it is about 3 hours and after 7-8 hours we noted slight decrease.

	SCN^-	CN^-	S^{2-}
Slope	$2.04 \cdot 10^{-4}$	$1.67 \cdot 10^{-4}$	$5.13 \cdot 10^{-3}$
R^2	0.9999	0.9986	0.9937
Linearity [ng]	150-2440	150-2440	150-2440

Analyte	*Repeatability [%]* (b)		*LOD [ng/mL]*		*LOQ [ng/mL]*	
	Intra-day	*Inter-day*	*3S/N*	*3σ/m*	*10S/N*	*10σ/m*
SCN^-	1.13	0.99	2.24	0.59	7.47	1.98
S^{2-}	6.2	5.2	0.12	1.24	0.38	4.14
CN^-	2.44	2.8	48.97	4.80	163.22	16.00

Protocol for saliva sampling and analysis

C1-C5 → non-smokers

C6-C10 → smokers

MIX-1/2/3 → mix saliva samples

Analysis of human saliva samples

50 µL of saliva sample 1:10

↓

10 µL NaOH + 100 µL $Et_3O^+BF_4^-$

↓

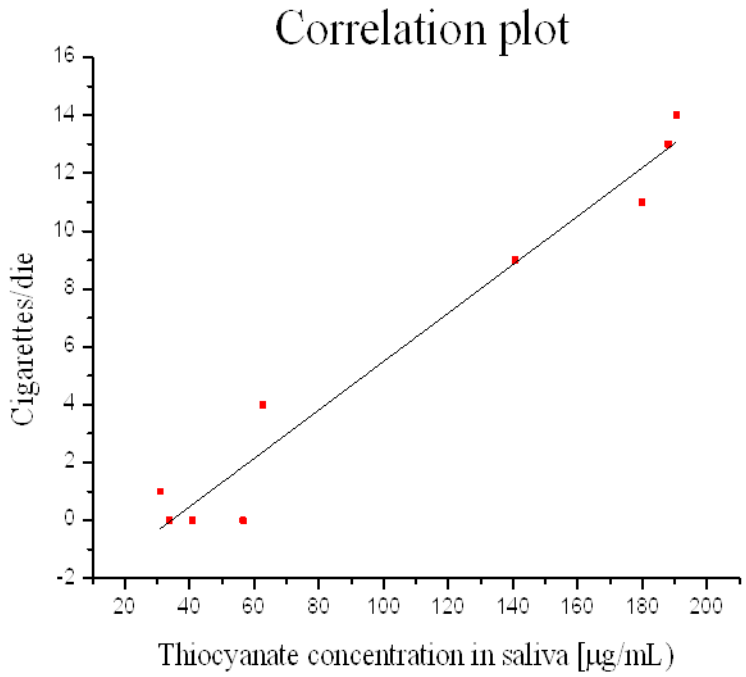
Internal standard: $S^{13}CN^-$

↓

HS/GC-MS

Sample	*Thiocyanate concentration [mg/L]*	
	I.S.Calibration plot	***Isotopic dilution***
C1	40.8 ± 0.3	41.3 ± 0.5
C2	56.5 ± 0.7	57.2 ± 0.7
C3	40.9 ± 0.4	41.7 ± 0.8
C4	33.8 ± 0.2	34.4 ± 0.3
C5	30.9 ± 0.1	31.2 ± 0.3
C6	62.7 ± 0.8	63.7 ± 0.5
C7	188 ± 1	191 ± 1
C8	140.7 ± 0.2	142.0 ± 0.5
C9	180 ± 2	182 ± 2
C10	190.6 ± 0.3	192.7 ± 0.8

The optimized method as been succesfully applied to the analysis of human saliva samples for the quantification of the thiocyanate. The use of the labeled internal standard ($S^{13}CN^-$) and the analysis of the headspace allow to eliminate, or reduce drastically, the matrix effect
Interesting observation (limited number of sample) is the correlation between the saliva Thiocyanate concentration and number of cigarettes smoked for day.



The work done at NRC will be pubished as scientific paper in few months

Determination of Thiocyanate in Human Saliva by derivatization with triethyloxonium tetrafluoroborate salt and Gas Chromatography-Mass Spectrometry

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ABSTRACT

In this work an analytical derivatization procedure has been successfully exploited, by salt of trialkyloxonium tetrafluoroborate $R_3O^+BF_4^-$ (R=Et), for determination and quantification of thiocyanate anion in human saliva samples. The study and the determination of the alkyl derivate (CH_3CH_2SCN) was carried out through analysis of head space (HS), thanks to the ability of trialkyloxonium salt to give organic volatile compounds, according with primary derivatization reaction $R_3O^+ + X^- \rightarrow RX + R_2O$. Before proceeding with the analysis of real samples, it was necessary to optimize the method of derivatization, in order to obtain maximum yields of reaction and avoid the formation of unwanted products. Samples were collected from ten different volunteers, with different ages, and stored in a refrigerator at -20°C. The alkyl derivate was analyzed by Gas Chromatography-Mass Spectrometry (GC-MS), equipped with head space autosampler and incubating tool. Under optimization condition, the calibration plot showed good linearity in the range of 9.530-4000 ηg/mL for SCN^- (R^2=1.000).