# SANIST-CIMS a tool for rapid and accurate product quality check

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Short title: SANIST-CIMS in analyte identification

Keyword: database search, compounds identification, bioinformatic, algorithm, EU law

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**Abstract**

The advent of new high-resolution mass spectrometry has opened new important frontiers especially in the profile analysis of molecular complex mixture. In fact, it is now possible to analyze thousands of molecular species per analysis. One of the most employed technique for this porpoises is Liquid Chromatography – Mass Spectrometry. Recently, the SANIST technological platform based on this technique integrated with Surface Activated Chemical Ionization/Electrospray (SACI/ESI) and a data elaboration system for compounds identification performed on the basis of EU directive (EU directive 2002/657/EC) has been developed. This technique provide benefits in terms of sensitivity increase due to the SACI/ESI ionization source and in the compounds identification precision increase that is performed by means of spectrum match criteria recognized by EU legislation. This technology can be used to check the composition quality in different fields: food, pharmacological and environmental. A SANIST limitation is that the existing methods makes possible to analyze low and medium/high molecular weight compounds only separately. Here we present a SANIST-CIMS approach that makes used of the in source Cloud Ion Mobility effect produced in SACI/ESI device to simultaneously analyze low and medium/high molecular weight compounds in a single run so to increase the throughput in the field of product control. The data obtained analyzing the qualitative composition of some commercial product has been shown and disclosed.

## Introduction

High resolution and mass accurate mass spectrometry (HR-MS) and its application [1-4] has known a strong diffusion in the recent years. The technology makes possible to analyze thousand compounds in a single runs and to provide information in their identities especially if combined to tandem mass spectrometry (MS/MS approach). These analytical performances has lead to a wide diffusion of this technique in the field of molecular mixture qualitative composition in food, pharmacological and environmental.[1-4]

HR-MS is employed to analyze both low molecular weight (MW) compounds (MW < 600 Da) and medium-high MW (MW > 600 Da) ones. Low MW could be typically metabolites,[5] contaminants, toxins and pesticides in food,[6-8] impurities in pharmacological products [9] etc. High molecular weight could be proteins,[10] polymers [11] etc.

A main limitations of the current mass spectrometric approach is that low and medium/high mass molecular species are processed separately due to the reduce the reduce the data complexity and increase the mass spectra quality.[12] The advent of Ion Mobility Mass Spectrometry (IM-MS)[13,14] has added another dimension to the analysis increasing the analytes discrimination level on the basis of molecular spatial conformations. The molecules are first ionized and then separated through gas interaction in the IM-MS mass spectrometry region. Cloud Ion Mobility Mass Spectrometry (CIMS)[15] is a high versatile ion mobility technology that makes possible toseparate low  from medium-high molecular weight species directly in mass spectrometer atmospheric pressure ionization source maximizing the ion transmission of the different species in the mass spectrometer vacuum region.

Here we propose a single run method based on SANIST technology [16,17] that incorporates SACI/ESI ionization source configured in CIMS[15] operative modality to execute a qualitative composition quality control of commercial products containin both low and medium/high MW compounds. This configuration permits to alternate the acquisition of the two compounds class in the same run alternating the voltage of the in source capillary and to differentially focalize in-source solvent cloud containing low MW and that containing medium high MW separately. The in source separation of MW

compounds class makes possible shows to reduce the matrix effect phenomenon [18] respect to the classical method based on LC-MS and using Electrospray (ESI). The reduction of matrix is maintained even lowering the gradient chromatographic conditions and this makes possible to further reduce the total run time.

The new method has been used to simultaneously test the presence of contaminants and allergen in food and pharmaceutical products. The resuts achieved using SANIST-CIMS were compared with these achieved by means of a LC-MS analytical system and disclosed.

## Materials and methods

*Chemicals*

Acetonitrile ($CH_3CN$) bi-distilled water, Dimethylsulfoxide (DMSO) and formic acid (HCOOH) were purchased from Sigma Aldrich (Milan, Italy). Fruit jounce were acquired by different producers and vaccines sample were achieved by Corvelva association (Padova, Italy)

*Sample selection, collection and preparation*

3 Juice fruits, 3 food supplement even to verify. and 3 vaccines samples were spiked with 1 microgram/microliter of Emoglobin and 1 mg/mL of Agilent tune mix as internal quality control standard. The samples were treated with 200 microliter of NH4HCO3 50 mmol buffer containing trypsin at a concentration of 2 ng/microliters. The samples were left at 37 °C overnight and in the last 30 minutes of digestion the temperature was increased to 57 °C as suggested by literature data.[20] The products were centrifuged at 13000 g for 10 minutes and 100 microliter of the surnatant were collected. The solid fraction were treated with 200 microliter of NH4HCO3 50 mmol buffer containing trypsin at a concentration of 2 ng/microliters. The digestion temperature and time conditions were the same previously cited. After the treatment the samples were centrifuged at 13000 g for 10 minutes and 100 microliter of the surnatant was added to the previous collected sample. The solid fraction was then dissolved using 200 microliter of Dimethylsulfoxide (DMSO). Each sample was prepared and analyzed in triplicate.

*Chromatography*

To analyze the selected analytes, an Ultimate 3000 UPLC (Thermo Fisher, San Jose, CA, USA) liquid chromatography apparatus was employed to obtain analyte separation before of mass spectrometric analysis. A Trascend System HPLC (Thermo Fisher, San Jose, CA, USA) was used for separation. The mobile phases were: A) H20 + 0.1% HCOOH and B) $CH_3CN$. A binary gradient was used: 2% of B was maintained for 2

minutes, in 7 minutes B was raised to 30%, in other 3 minutes B was brought to 80% and maintained for 0.9 minutes, then 2% of B was reached in 0.1 minute and the column was re-equilibrated in starting conditions for 4 minutes. The chromatographic flow was 0.50 mL/minute. The injection volume was 20 µL.

*Mass spectrometry*

Product analyses analysis were performed using LTQ XL ORBITRAP mass Spectrometer (Thermo Fisher, San Jose, USA) coupled to Heated ESI a Heated SACI-ESI source (described in [1]) and operated in CIMS alternate mode. [15]

Heated ESI capillary voltage was 2750 Volt, dry gas: 2L/min, Nebulizer: 60 psi and Temperature: 40°C. Tandem mass spectrometry experiments were performed in Collision Induced Dissociation (CID) conditions using He as the collision gas using a collision energy of 35% of its maximum value (5 V peak to peak).

Heated SACI-ESI was set in alternate CIMS conditions so to focalize ion cloud containing low molecular MW and medium/Hight one. The former conditions were: capillary voltage was 100 Volt, SACI surface voltage was 47 V, Dry gas: 0.5L/min, Nebulizer: 70 psi and Temperature: 40°C. The later conditions were: capillary voltage was 1500 Volt, SACI surface voltage was 47 V, Dry gas: 2L/min, Nebulizer: 70 psi and Temperature: 40°C.

Tandem mass spectrometry experiments were performed in Collision Induced Dissociation (CID) conditions using $N_2$ as the collision gas.

*SANIST data elaboration platform description*

SANIST data elaboration platform is composed by a microcomputer remote station connected by an ethernet cable to the main workstation that acquires the data and collects the spectra to be identified. The MRM spectra database is stored on the microcomputer together with the data elaboration algorithm. MSP data format was employed. A SAMBA server was employed to share the MSP MRM spectra of the compounds to be characterized. When the MSP spectra are saved in the shared folder, the microcomputer automatically executes the EU match algorithm and generates a

progressive report in ASCII format containing the spectral match and the match results in terms of identification confidence.

*SANIST spectral matching algorithm*

DrugDisc algorithm is based on a variation of the Geometric Čebyšëv distance formula (i):

(i) $dI,Ir=max_i|Ii- Iri|$

where I and Ir are the detected ion intensity associated to a specific m/z sample fragment and to the reference spectrum acquired on the laboratory instruments respectively. To adapt to the EU directive calculations [8] the formula (I) has been enhanced obtaining formula (II):

(ii) $dI,Ir\%=max_i|Ii- Iri||Iri|*100$

The d(I,Ir)% variance is evaluated following the EU directive reported in Table 1.

**Results and discussion**

Preliminary data were achieved by setting CIMS [15] cloud ion mobility to discriminate among low (MW < 600 Da) and medium molecular weight (MW 600 – 4000 Da) analyte in the same LC-SACI/ESI-MS MS/MS analysis. The method was set so to switch among low and high molecular weight each 0.2 sec. The ion cloud droplet containing low molecular weight analyte were selected by setting the ion mobility nitrogen gas to 0.5 L/sec and acting on the cloud focusing voltage (150 V) to selecting the solvent clouds containing the low m/z singly charged species. As disclosed in the paper of Arzoni et. Coworckers [15] the ion mobility nitrogen gas under the 3 L/min do not select any charged cloud and every cloud containing different molecular weight and charged analytes are focalized vs the first mass spectrometer ion vacum region at 0.47 tor. The solvent cloud containing the low m/z ratio analytes are selected using the 150 V focusing voltages. In fact, in these conditions the solvent cloud containing the low singly species are better focalized to the MS vacum region while the focusing voltage is not optimal to efficiently direct the high m/z in the MS entrance hole. The focalization of solvent ion cloud containing high m/z species occurs at 5 L/min of ion mobility nitrogen gas and at a focusing voltage of 600 V. The higher value of ion mobility nitrogen gas contribute to defocalize the solvent clouds containing low m/z ratio and a lower ion resultant charged state while the higher focusing voltage selectively increase the focalization efficiency of the solvent cloud containing high m/z species.[15]

Figure 1 shows the product quality control method for data sample preparation, data acquisition and elaboration. Basically, the product is dried under nitrogen stream. After that a triple solvent extraction using water, methanol and acetonitril was performer. The proteins container in the water fraction are digested using tripsins. The washing fraction are collected and analyzed by means of SANIST-CIMS. Low and high m/z ions are alternatively acquired using CIMS effect follower by MS/MS fragmentation in data dependent Scan mode to acquire the tandem Mass spectra. Figure 2a and b shows the SANIST-CIMS Mass chromatogram acquired in both positive and negative acquisition mode. A Mass chromatogram region has been Aldo highlighted to show the varius steps of the analysis. In particular, the full Scan regional obtained by analyzing the solvent charged Cloud containing low and high m/z analyzed together with data dependent fragmentation region are clearly shown. The obtained files are then converted to the

mzXML universal format to be analyzed by means of the SANIST data elaboration tools. Low m/z ratio identity is obtained by combine the accurate m/z search with MS/MS spectrum match. The identity of the obtained candidates is confirmed using the SANIST algorithm [17] based on the European Union (EU) directive (EU directive 2002/657/EC). The high m/z ratio are subjected to peptide mass mapping, database search and denovo sequence to identify the protein fraction. ToxPro database is used to identify toxins originated by bacteria at other sources. If a toxins or dangerous compound Is detected It presence Is confirmed by means of the corresponding analytical standard. Even in this case the MS/MS spectrum match is performed following the EU directive (EU directive 2002/657/EC) [17]. A final report is obtained joining the two obtained dataset.

The developer SANIST-CIMS method has been so employed to verify the composition quality of the follow commercial products:

a) 3 juice fruits.
b) 3 food supplement even to verify.
c) 3 Commerciale vaccines.

In the case of juice fruits the results of the analysis are shown in tablet 1. As It can be seen different pesticides and toxins have been detected. However, no allergen have been identified.

The data obtained analyzing food supplements are reporter in table 2. Particular relevant was the case of supplements 1. The product is commercialized for its proprieties related to maintain an optimal body weight. However, a compounds similar to a chlorinated benzodiazepine was detected in negative acquisition mode. The isotopical analysis performed in the elemental composition screening phase clearly sows the presence of three chloride insider the analytical structure. The fragmentation spectrum suggests a structure similar to that shown in scheme 1. However, the identification analysis do not confirm exactly the identity on the basis of the EU directive (EU directive 2002/657/EC) [17]. The detected compounds is so probably derived by modified form of a benzodiazepine.

Concerning the vaccines the results are clearly shown in table 2. As It can be observed some contaminants have been detected in the vaccines. Most of them have not been confirmed considering the EU directive. Other substances that have not been

subjected to confirm check are reported in supplementary materials (Table S1). However, a fragmentation similarity with the screened compounds is present. All the contaminations can be potentially originated from the biological material employed to produce vaccines. Interesting, is the absence of the antigens in vaccine 1. This fact potentially make, the product, ineffective.

## Conclusions

The SANIST-CIMS technology provide strong benefits in terms of total run time, precision, accuracy and cost in the product quality screening and confirm check. The platform can be so useful to different companies and public control institutes to check the data quality and product contamination and adulteration.

**Acknowledgements**

The study was supported thought Corvelva association granting funding.

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**Captions**

**Figure 1:** product quality control method for data sample preparation, data acquisition and elaboration.

**Figure 2:** SANIST-CIMS Mass chromatogram acquired in both a) positive and b) negative acquisition mode.

**Table 1:** Jouice fruits the identification results.

**Table 2:** Data obtained analyzing food supplements.

**Table 3:** Vaccines identification results.

**Table 1:** Juice fruits the identification results.

| ID | Product | Compounds | Classification | EU test result |
|---|---|---|---|---|
| 1 | Juice fruits | glyphosate | Pesticide | Positive |
| 2 | Juice fruits | glyphosate | Pesticide | Positive |
| 3 | Juice fruits | glyphosate | Pesticide | Positive |

**Table 2:** Data obtained analyzing food supplements.

| ID | Product | Compounds | Classification | EU test result |
|---|---|---|---|---|
| 1 | Suplement | Tri-chlorurated compound | High abundant contaminant | Negative |
| 2 | Suplement | No contaminant | No contaminant | Positive |
| 3 | Suplement | No contaminant | No contaminant | Positive<br>Not confirmed with std<br>Negative<br>Negative |

**Table 3:** Vaccines identification results.

| ID | Product | Compounds | Classification | EU test result |
|---|---|---|---|---|
| 1 | Vaccine | Antigen | Antigens | Negative |
| | | Different aminoacids | Contaminant | Negative |
| 2 | Vaccine | Antigen | Antigens | Positive |
| | | APDB | Contaminant | Not confirmed with std |
| | | Mycocyclosin | Contaminant | Negative |
| | | Cephalexin monohydrate | Contaminant | Negative |
| 3 | Vaccine | Antigen | Antigens | Positive |
| | | Differents aminoacids | Contaminant | Not confirmed with std |
| | | Erastin | Contaminant | Negative |
| | | 5-Methyltetrahydrofolate | Contaminant | Negative |

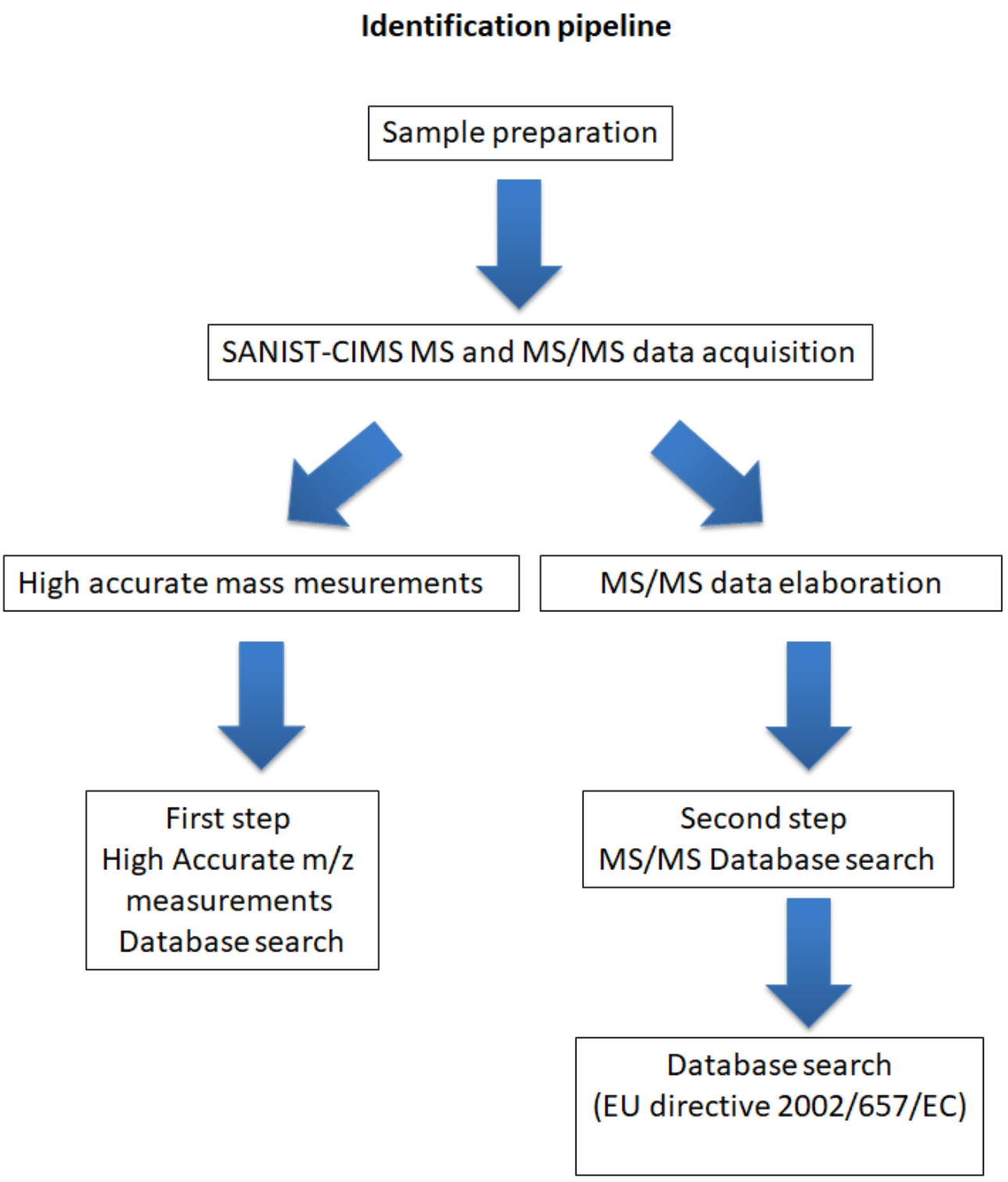
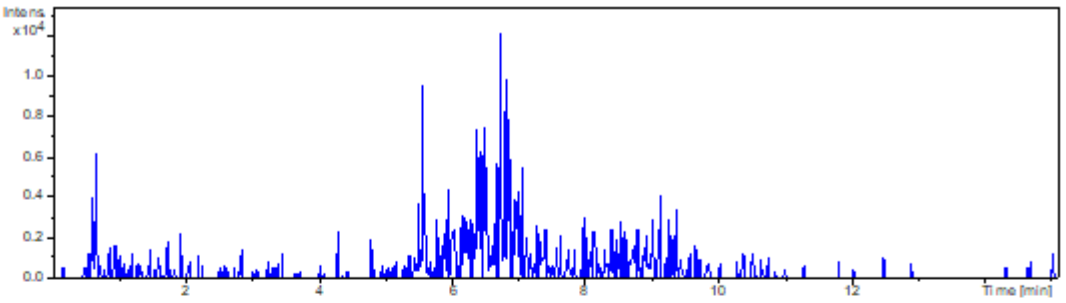

Identification pipeline
Sample preparation
SANIST-CIMS MS and MS/MS data acquisition
High accurate mass mesurements
MS/MS data elaboration
First step
High Accurate m/z
measurements
Database search
Second step
MS/MS Database search
Database search
(EU directive 2002/657/EC)

**Figure 1**

a)


Intens.
x10^4
1.0
0.8
0.6
0.4
0.2
0.0
2
4
6
8
10
12
Time [min]

b)

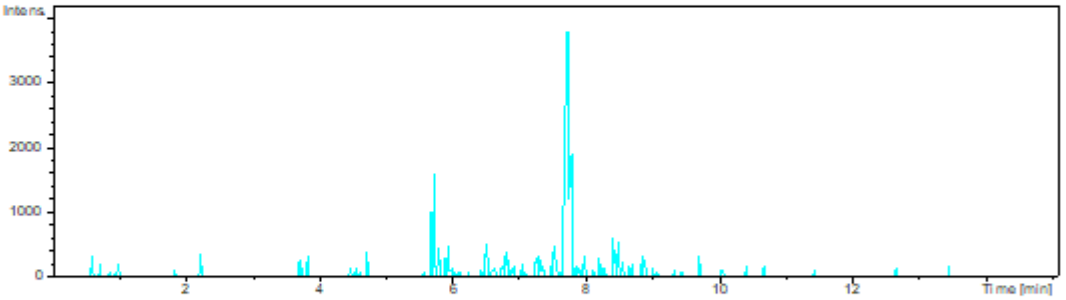

Intens.
3000
2000
1000
0
2
4
6
8
10
12
Time [min]

**Figure 2**