Gas Chromatography-Ion Mobility Spectrometry Instrument for Medical Applications: A Calibration Protocol for ppb and ppt Concentration Range

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Jorge M. Fernandes[1,2], Valentina Vassilenko[1,2], Pedro C. Moura[1] and Viktor Fetter[3]

[1] Laboratory of Instrumentation, Biomedical Engineering and Radiation Physics (LIBPhys-UNL), Department of Physics, NOVA School of Science and Technology, NOVA University of Lisbon, 2829-516 Caparica, Portugal
[2] NMT, S.A., Edifício Madan Parque, Rua dos Inventores, 2825-182, Caparica, Portugal
[3] Airbus Defence and Space GmbH - Space Systems, Dept. TESXS Science Engineering, 88046 Friedrichshafen, Germany
j.manuel@campus.fct.unl.pt

Abstract. Medical diagnosis research is driven into the development of non-invasive diagnosis devices centered in fast and precise analytical tools and instrumentation. This led to Volatile Organic Compounds (VOCs) being identified as metabolomics biomarkers for several diseases, including respiratory infections, cancer and even COVID 19 non-invasive test. While VOCs give a direct access to physiological states, their applicability requires detections at low concentration ranges (ppb_v-ppt_v). However, its clinical success is strongly dependent on precise and robust calibration methods. In this work we describe a calibration protocol of volatile organic compounds in low concentration range (ppbv-pptv) for analytical GC-IMS technology which offer a quick in-situ results in medical diagnosis. The calibration is based on permeation tubes which are monitored using thermogravimetric methods to estimate mass loss ratio over time establishing emitted concentrations. Notwithstanding future improvements, herein calibration methodology results are a promising step forward in medical diagnosis and applications.

Keywords: Metabolomics, Volatile Organic Compounds, Medical Diagnosis, Analytical Techniques, Calibration, Automated Systems, Artificial Intelligence

1 Introduction

Modern medicine relies on innovative technology breakthroughs that enable to identify different diseases and physiological states rapidly, accurately, and effortlessly. Thus, research appears to be shifting its route towards techniques that permit rapid analysis of biological samples at low-cost, with reliable results and, mainly, non-invasively. Consequently, systems of identification and quantification of compounds described as pathological biomarkers are increasingly desired.

Ion Mobility Spectrometry (IMS) has been asserting itself as one of the most promising and adequate analytical technologies to fulfil the contemporary necessities of medicine [1]. The excellent reputation IMS has been gaining in latest years is due to

its extraordinary detection limits of volatile organic compounds (VOC), specifically, in low ppb_v (parts per billion by volume) or ng/l (nanograms per litre) of concentration ranges or even in ppt_v (parts per trillion by volume) or pg/l (picograms per litre) [2]. Similarly, its analytical flexibility, real-time monitoring and low-cost, as well as, its high selectivity and sensitivity when coupled with Gas Chromatography (GC) technique, contribute to affirm GC-IMS as one of the most important and useful portable analytical instrumentation for health applications [3] [4]. GC-IMS operates by creating ions from any volatile organic compound generally ionised by a Tritium radiation source, which pass through the IMS drift tube due to a weak but homogenous electric field after having been pre-separated inside a chromatographic column [5] [6]. A more detailed description of the IMS principle is given elsewhere [7].

IMS outstanding sensitivity combining with its selectivity and ability to deliver results promptly facilitates the rapid characterization of biological samples and detection of biomarkers [8]. A biomarker is an indicator of, not only, physiological states, but also, pathological conditions. The blood stream has dozens of distinct volatile organic compounds resulting from these endogenous activities and once emitted, analysed, and identified, they represent a non-invasive, rapid, painless, and economic door to human being's health assessment [9] [10]. The emission can occur via skin [11], fluids, and even exhaled breath [12]. A considerable number of diseases was already been correlated to specific biomarkers, and they belong to an extensive spectrum that includes conditions, such as, smoking-habits identification [13], asthma and diabetes [14], and even concerning conditions like lung or breast cancer [15]. Nevertheless, endogenous volatile compounds can still function as a biomarker for unnormal situations accordingly with their concentrations (low ppb_v). Even if a compound is frequently found in a regular basis, its distinct concentration from ordinary levels, may indicate physiological alterations or abnormality. Hence, not solely identification, but also VOC quantification have an extreme relevance in defining a biomarker, however, scientific research for precise VOC quantification and efficient calibration method are scarce and often incomplete. Therefore, developing an efficient and precise calibration method for GC-IMS calibration would be a big step for both IMS and medical diagnosis.

Herein we propose a calibration methodology of a GC-IMS devices based on permeation tubes to generate low VOC concentrations (ppb_v) by a thermogravimetric approach. The generation of gas standards from pure substances requires the creation of precise and repeatable concentrations and many methods have been developed for gas generation, which are divided into two categories: static and dynamic [16] [17] [18]. Static methods include gravimetric, partial pressure and volumetric approaches, and flexible, single and multiple rigid chamber, whilst dynamic methods include six types: injection, permeation, diffusion, evaporation, electrolytic and chemical [16] [17].

Permeation tubes are a dynamic method with an advantage over other diffusive and static techniques, they can generate low concentrations more stable over time. The basic design of a permeation tube or source is a liquid or gas-filled tube of PTFE or other semi-inert permeable material which is temperature-controlled (Fig.1) [18]. Inside a permeation tube, a chemical can permeates through its walls at a constant rate for a given temperature; then its vapor mixes and is carried by a diluent, or a make-up flow into an analyser [16] [17] [18].

2 Relationship to Applied Artificial Intelligence Systems

The calibration protocol presented here combined with a previously published work on the development of an algorithm for automatic peak detection and quantification of GC-IMS Spectra, establishes a project to develop an automated tool for qualitative and quantitative identification of VOCs in low concentration (ppb_v/ppt_v) [7]. Such tools aim to improve the applications and results from GC-IMS analysis in air quality, space exploration and human health. As such, it is crucial to develop and implement an intelligent algorithm alongside with the GC-IMS technology, which is capable of identify and, more importantly, assess the concentration levels of organic compounds.

Our development of a precise and effective calibration protocol not only improves the current position of IMS calibration methodologies but is also, the next phase in the development and improvement of our previously mentioned algorithm. This algorithm was qualified to identify and quantify VOC compound signals in GC-IMS spectra. However, it lacked the ability to establish its relationship with a concentration value, which is crucial for some clinical applications. With the development of a calibration protocol this issue can be addressed and solved, therefore, improving the algorithm and enable it to evaluate potential risks by its own means. This relies not only on our calibration protocol already established but also on a large and diversified dataset of samples and a big compound library which are currently both currently being built.

Using neuronal networks, the current algorithm abilities can be enhanced in relation to compound identification, risk assessment and in establishing disease biomarkers. Also, several IMS data processing methods, as denoising (e.g., wavelets), scaling (min-max scaling), baseline correction, supervised (e.g., genetic algorithm) or unsupervised analysis, such as Principal component analysis (PCA) or cluster analysis, can benefit from machine learning and artificial intelligence implementation. Moreover, machine learning can improve collected information from GC-IMS data, as well as the modulated linear or logarithmic regression involved in establishing a calibration curve and the resulting estimation of VOCs concentration, which will inevitably benefit both medical instruments related to IMS and medical diagnosis.

3 Materials and Methods

Our proposed calibration protocol consists in creating permeation tubes containing a pure volatile organic compound and continuously weighting it inside a hoven at constant temperatures by thermogravimetry methods over several days. Adjusting a linear regression to tube mass loss permits to estimate an emission rate from its slope. Each temperature will generate different mass loss ratios therefore creating different concentration by simultaneous varying the hoven make-up flow and thus, after applying equation 1 the pure gas concentration can be determined [16] [19].

$$C = \left(q_d \times 22.4/_M\right)/Q\ , \tag{1}$$

In equation 1, C is the concentration in ppm, q_d the permeation ration ng/min, M the compound's molecular weight in g/mol, and Q the flow ratio in mL/min.

3.1 Instrumentation and Selected Compound

A BreathSpec® device from GAS Dortmund was used for the developed calibration method. This instrumentation consists of an Ion Mobility spectrometer coupled with Gas Chromatography (GC-IMS) which used an MXT-200 column, of 30 m length and 0,53 mm internal diameter coated with a 1 μm thickness mid-polar stationary phase of trifluoropropylmethyl polysiloxane. Whereas this IMS instrumentation uses a Tritium, 3H (β-radiation: 300 MBq) ionisation source, a drift tube length of 98 mm with a 5-kV switchable polarity and an electric filed strength of 500 V/cm. Purified air was used as a carrier and drift gas which was filtered by a device coupled with the GC-IMS, named Circular Gas Flow Unit (CGFU) from GAS Dortmund.

A LABSYS evo TGA 1150® device from Setaram instrumentation was employed for thermogravimetric analysis of four permeation tubes, which has a temperature range from room temperature to 1150ºC, a weighing precision of 0.01 % with a resolution of 0.2 μg; 0.02 μg and uses purified air as its flow gas.

The selected compound was 2-hexanone, purchased from Sigma-Aldrich with +99% purity and utilized to assemble four permeation tubes generating several standard gas concentrations.

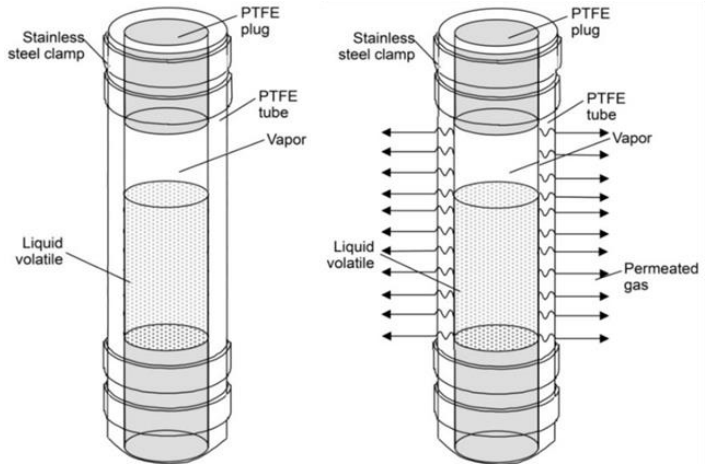

Fig. 1. A generalize schematic of permeation tubes including its components [18].

3.2 Calibration Systems and Procedure

Four permeation tubes were made by depositing 0.2 mL of 2-hexanone in a 1/4" PTFE tubing with a length of 2 cm. Tube extremities were sealed with 5mm PTFE end caps of 0.5 cm length which were lock in place with 0.5 cm length mild steel end crimps pressed by a crimping vise (Fig.1). Tube effective length, or the distance between the two interior end plug surfaces, was 1 cm. Materials for the permeation tube were fabricated by Owlstone Inc. and are available as a Permeation Tube Manufacturing Kit.

The calibration systems were comprised of a CGFU couple with GC-IMS connected in its sample inlet to the TGA device by a Teflon tube of approximately 40 centimetres.

The CGFU was responsible for purifying the drift and carrier gas into the GC-IMS. The GC-IMS is a gas analyser and the device to be calibrated. Whilst the TGA device provided accurate mass loss ratio from the permeation tubes, temperature control and flow into the sample inlet. Temperature values used for creating accurate concentration were 40, 60 and 85 ºC and the flow values included 25, 50, 100, 150 and 200 mL/min. Measurements were taken each 15 min after a stable mass loss ratio was achieved, and for each concentration value 5 replicates were made, while each gas flow change was interspersed by 15 min before any measurement was performed.

4 Results and Discussion

Several conditions and parameters were examined and analyzed leading to the creation of a calibration protocol and characterization of a calibration curve (Fig.2). Our proposed calibration protocol is defined by three main phases: (i) permeation tube construction and filling; (ii) estimating emission rate of the permeation tube by thermogravimetry analysis; (iii) generating several concentrations from each tube emission by changing flow rate. In phase one, during the construction of the tube, it was important to keep their dimensions as similar as possible and strongly crimping their extremities to avoid any liquid leakage.

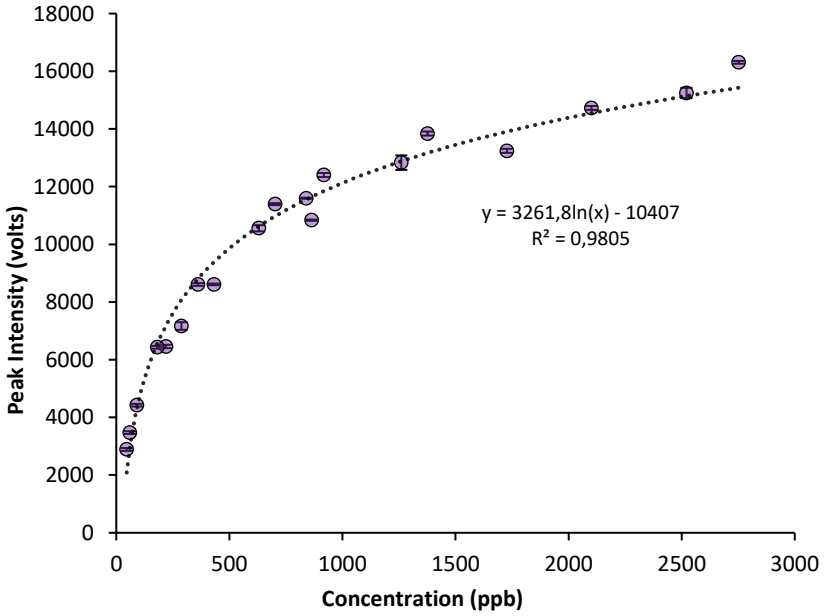

Fig. 2. Logarithmic regression for peak intensity (volts) per concentration (ppb).

Once constructed and filled, the tubes were left to stabilize for 24 hours. Afterwards a tube was placed inside the TGA device's hoven and set at a desired temperature for approximately 5 days, considering the amount permeating a tube is relative to the compound inside it and the hoven temperature. The thermogravimetric device would

control temperature and weigh constantly the tube's mass, producing a decreasing graphic with a linear behavior. When a stable emission was achieved a linear regression was adjusted to this graph estimating its slope. Afterwards, GC-IM measurements were analyzed, calculating an intensity value for 2-hexanone from its two occurring peaks. Finally, a plot was constructed with concentrations and respective intensities to which a logarithmic function was adjusted. Hence, results provided an expression to determine peak intensity from concentration values, known as a calibration curve (Fig.2).

Table 1 shows concentration, values mean intensities and standard deviations used to develop our presented calibration curve (Fig.2). Generally low standard deviations were observed from measurements indicating an elevated stability and reputability from the GC-IMS. The lowest concentration created was 46 ppb, however when estimating the GC-IMS limit of detection (LOD), a value of 26 ppb was observed.

Emission rates from tube 1, 2, and 3 are 615, 193, 564 and 126 ng/min respectively and the calibration curve is expressed by equation 2 having an R^2 of 0.98.

$$y = 3261{,}8 \times \log(x) + 12125 \qquad (\mathbf{2})$$

Table 1. Intensity and standard deviations of four permeation tubes and estimated concentration

Concentration [ppb]	Mean Intensity (volts)	Standard deviation
Permeation tube 1	----	----
2753	16309,60	47,94
1376	13840,21	70,28
918	12403,41	70,42
702	11394,99	33,99
Permeation tube 2	----	----
1727	13241,45	69,39
864	10837,40	21,90
432	8611,60	28,27
288	7168,54	133,17
220	6458,79	58,55
Permeation tube 3	----	----
2521	15247,31	177,88
2101	14727,63	67,01
1260	12832,12	250,80
840	11598,73	18,36
630	10557,16	107,10
Permeation tube 4	----	----
362	8607,41	46,32
181	6428,60	48,64
91	4424,19	45,90
60	3473,57	47,66
46	2888,47	49,69

5 Conclusions and Future Work

A calibration protocol was established using the dynamic method of permeation tubes by controlled temperature and carrier flow with a thermogravimetry device. Three main phases were defined in the protocol which allowed to create a concentration range from 2700 to 46 ppb. Using the developed protocol, it was possible to calibrate a GC-IMS device for 2-hexanone attaining a logarithmic calibration curve, $y = 3261{,}8 \times \log(x) + 12125$, with an R^2 of 0.98.and an estimated LOD of 26 ppb.

During protocol development it became evident certain parameters and conditions were crucial for developing an accurate calibration curve. Temperature control was essential in the calculation of an accurate emission rate, whilst this emission rate should be conducted in a period of 4-5 days or more. Whereas for IMS measurements it was essential to allow an interval between flow changes and their respective samples. Nonetheless, if the proper conditions are established, calibration is a straightforward procedure which can be improved and automated by implementing machine learning or artificial intelligence to processing thermogravimetry and GC-IMS spectra data. Moreover, this same protocol can be used for the calibration of VOCs in ppb and ppt ranges of concentration. Calibration of GC-IMS devices in low ppb is a crucial point in implementing it in medical diagnosis or medical devices, and its improvement by automated tools further expands its potential and applicability in the field of medicine.

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