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A methodological approach to the selection of liquid reagents for chemical ionization ion trap-gas chromatography mass spectrometry: A case study of GBL and 1,4-BD

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ABSTRACT

A new approach is proposed for the selection of reagent ion species in a gas chromatography-chemical ionization mass spectrometry (GC-PICI-MS) method for GBL and 1,4-BD determination, the two "pro-drugs" of gamma-hydroxybutyric acid (GHB), a drug associated with sexual assault. The GC-PICI-MS is often the best technique to avoid an extended fragmentation occurring in EI source and it preserves the information on molecular ions. Ion-trap mass spectrometry (IT-MS) is a valuable tool in chemical ionization experiments, commonly affording reaction times 10^4–10^5 higher than those in conventional CI sources. This feature allows the use of either vapors from liquid reagents, or many reactant species that are difficult to generate and employ in the conventional CI experiments. In this research acetone, acetonitrile, methanol and diethylamine were evaluated to generate vapors of the chemical ionization species. The use of liquid CI reagent offers a wide range of chemical–physical properties that can greatly affect the specificity, with the possibility to modulate the detection of the analyte in comparison with background or matrix interferences. The experimental data using different CI liquid reagents and reaction times were compared through calibration curves of GBL and 1,4-BD (ranging from 10 to 1000 ng/mL). The linear regression curves obtained were used to calculate the sensitivity (slope) and limit of detection (LOD) of the method. Methanol resulted in the most efficient reagent for the determination of studied analytes. However, its employment as ionization agent of the 1,4-BD favors the hydride abstraction mechanism or hydrogen loss from protonated-molecule ions. These phenomena can be considered as a possible sources of uncertainty or errors. Therefore, acetonitrile can be employed as a good compromise between sensitivity and reliability of signal for both analytes.

1. Introduction

Mass Spectrometry (MS) is a well recognized, highly sensitive and selective tool for analysis in many fields. The easy coupling with gas chromatography (GC) offers a third-dimension separation feature (i.e. mass spectrum) allowing the detection of analytes in complex matrices even at ultra-trace levels [1]. In benchtop GC–MS, the electron ionization (EI) source is the most common ion generation system. This ionization source, at a given electron energy, produces a characteristic and reproducible mass spectrum which allows a tentative compounds identification through the comparison with spectral databases.

However, in some cases, EI mode is not always the most suitable ionization method since the associated extended analyte fragmentation may lead to a loss of information concerning the molecular ion [2]. As a matter of fact, the molecular weight evaluation of analytes is often carried out using chemical ionization (CI), which allows a better control of the internal energy deposited during the ionization processes [1]. In addition, selectivity and/or sensitivity can be increased, for selected compound classes, by using suitable CI reagents with different proton affinities and ion-molecule formation properties [3].

Generally, the CI mechanisms involve bimolecular processes during analyte ions generation. The occurrence of bimolecular reactions requires a sufficiently large number of ion-molecule collisions during the dwell time of the reagent species in the ion source. This is generally achieved by largely increasing the partial pressure of the CI reagent gas in the ionization chamber. In any CI plasma, both positive and negative ions are present, e.g., $[M+H]^+$ and $[M-H]^-$ ions (albeit in different amounts), and it is just the polarity of the

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acceleration voltage which determines the extraction of positive or negative ions from the ion source [4].

The positive-ion chemical ionization (PICI) is a multi-step process that involves different reactive ionic species. In the first step, a reagent "R" is ionized by interaction with the electrons emitted by the filament, allowing the formation of $[R]^{+\cdot}$ ions. In the following step, $[R]^{+\bullet}$ interacts with the R neutral molecules, present in the ionization source in large excess, leading to the formation of other reacting species, such as $[R+H]^+$. In the last step, the analyte reacts with ionic species present in reagent plasma. Common reactions occurring in the last step of the PICI process are [5]:

(A) Protontransfer : $[RH]^+ + M \rightarrow [MH]^+ + R$

(B) Hydrideabstraction : $[R]^+ + M \rightarrow [M-H]^+ + RH$

(C) Association : $[R]^+ + M \rightarrow [MR]^+$

(D) Chargetransfer : $[R]^+ + M \rightarrow [M]^+ + R$

Most of the common reagents show the proton transfer as the major reaction, leading to the formation of the protonated analyte ions. On the other hand, the energy involved in the CI ionization mechanisms can be enough to activate the fragmentation processes of the $[M+H]^+$ species with consequent increase of the complexity of the CI mass spectrum.

Traditional CI reagents are gases in standard conditions (i.e. methane, isobutane, ammonia) endowed with some evident drawbacks: the need of high pressure cylinders and pressure regulation devices, risk of explosions with flammable gases (i.e. methane, isobutane), limited choice of a suitable reagent, high management costs, etc. Many of these problems could be overcome employing vapors from liquid reagents. In fact, in this case the choice of a suitable reagent is greatly enhanced since there are many compounds available with respect to gases, and the chance to find a suitable reagent is higher. In addition, the limited amounts needed avoid the toxicity problems or safety risks.

In the present work, the ionization features of the *"in situ"(ionization)* ion trap mass spectrometer (*in situ* IT-MS) were evaluated. This instrumental configuration enables the trapping of ions for a relatively long time (tens of milliseconds) thus increasing tremendously the probability of interaction with neutral molecules present in the surroundings [1]. Therefore, it is possible to obtain a CI reaction plasma with only ppm concentrations of reagent gas, so that also liquid reagents (with rather low vapor pressure) can be employed as such.

Gas chromatography coupled with mass spectrometry (GC–MS) and CI have been widely used for decades to determine small quantities of drugs in multiple matrices. This is the case of gamma-butyrolactone (GBL) and 1,4-butanediol (1,4-BD), the two "prodrugs" of gamma-hydroxybutyric acid (GHB). They are drugs of abuse often associated with drug-facilitated sexual assault (DFSA) [6]. After administration, GBL and 1,4-BD are enzymatically converted to GHB in many tissues, with comparable biological effects and risks [7–10]. GHB and GBL have been recently subjected to legal constraints in many countries, while 1,4-BD is not included in any prohibited substances list and is commercially available as industrial solvent [11].

Qualitative and quantitative determination of GHB and GBL in various matrices has been carried out employing GC-PICI-MS, often using methane as CI reagent [12]. In many published methods, the procedure takes advantage of the quantitative conversion of GHB into GBL by dehydration; such reaction can occur both at high temperatures and in acidic conditions [13–16]. Recently, a method for quantitative determination of GHB, GBL and 1,4-BD in dietary supplements using GC-PICI-MS was published [17]. In this research, full conversion of GHB to GBL was achieved in the gas chromatograph injection port and a comparison between the EI and PICI mass spectra of GBL was made. In order to preserve the information derived from molecular ions, with the intrinsic improvement in both specificity and sensitivity, CI was selected for the quantitative analysis and acetonitrile was chosen as CI reagent.

The aim of this work is the evaluation of the analytical performances, employing different liquid CI reagents, using the GC-PICI-MS method for the determination of GBL and 1,4-BD as case study. The influence of parameters, such as the nature of the liquid reagent (different proton affinity) and reaction time (different yield of CI-reaction), on the analytical results was studied.

The choice of the suitable CI reagent is of primary importance on the analytical results such as quality of spectra or ionization yield. In fact, the thermodynamics of the reagent-analyte interaction determines the amount of the energy deposited, and consequently, the degree of fragmentation of the analyte along with all the related information regarding the molecular ions species (e.g. $[M+H]^+$, $[M-H]^+$, etc.). Moreover, the choice of the reagent gas can be finely tuned and could greatly affect specificity, with the possibility to modulate the ionization of the analyte with respect to the matrix interferences [18]. For instance, if only the proton transfer mechanism is considered, the yield of reaction largely depends on the different proton affinities of analyte and CI reagent [5]. On the other hand, different reagent species could be present in the reagent plasma leading to different ionization mechanisms. Therefore, many solvents commonly used in laboratory can be used as CI reagent to afford a wide range of proton affinities [19] and many other potential ionization species generated in the *in situ* IT-MS.

In the present work, the effect of four liquid reagents, acetonitrile, methanol, acetone and diethylamine, was investigated. This selection was motivated by both their boiling points (82, 65, 56 and 55 °C, respectively), so as to obtain a suitable vapor pressure for CI, and the range of proton affinities, that were similar to those of the analytes (Table 1). Diethylamine was chosen as a high-end reference for high proton affinity with respect to the analytes.

The effect of different reaction times between the analytes and ionic reagent species was also investigated.

2. Materials and methods

2.1. Chemicals

GBL was purchased from Merck (Milan, Italy). 1,4-BD, acetonitrile, methanol, acetone and diethylamine were all supplied by Sigma–Aldrich (Milan, Italy).

2.2. Instruments

The GC-PICI-MS analysis was performed by a Varian CP-3800 gas-chromatograph coupled with a Saturn-2200 ion trap mass spectrometer (Palo Alto, CA, USA) equipped with multiple CI reagent module. Chromatographic separations were performed by a Zebron ZB-WAX capillary column (30 m × 0.25 mm i.d., 0.25 μM

Table 1
Proton affinities of GBL, 1,4-BD and the CI liquid reagents data given from E.P.L. Hunter and Lias [19].

Compound	Proton affinity (kJ mol^{-1})
GBL	840.0
1,4-BD	915.6
Methanol	754.3
Acetonitrile	779.2
Acetone	812.0
Diethylamine	952.4