IDENTIFICATION OF NITROUS OXIDE FOR FORENSIC PURPOSES

Georgi Bonchev

Laboratory of Analytical Toxicology
Military Medical Academy, Naval Hospital – Varna

ABSTRACT

During the last two years the number of reported nitrous oxide (N_2O) self-poisoning cases in Northeastern Bulgaria are constantly growing up. Accordingly, there is a substantial increase in demand for analytical toxicology to provide help in fighting the illicit N_2O sales and distribution. Although laboratory confirmation of nitrous oxide intoxication in humans is an extremely difficult task, identification of nitrous oxide in physical evidences found at crime scenes proved to be easily achievable. An ultra rapid gas chromatography – mass spectroscopy approach for reliable N_2O confirmation, applied for providing irrefutable evidences in crime investigations, is discussed.

Keywords: *nitrous oxide, laughing gas, forensic science, physical evidence*

INTRODUCTION

Nitrous oxide (N_2O) is a colourless non-irritating gas with a sweetish odour, roughly 1.5 times heavier than air. Although non-flammable, it supports combustion due to its oxidizing properties at high temperatures. At usual conditions its reactivity is considered very low and it is routinely described as almost chemically inert.

Due to its both anxiolytic and analgesic properties, it has played a significant role in medicine, mostly dentistry, as inhalational anaesthetic. Retrospectively, N_2O, along with diethyl ether and chloroform, is one of the first medicinally recognized agents for surgical anaesthesia [1]. In modern anaesthesiology nitrous oxide is still used (and in some cases preferred) as a single agent in dental interventions and minor surgery [2, 3]. As it is the weakest known inhalational anaesthetic, in general surgery nitrous oxide is used only as adjuvant in combination with more potent agents. Its medicinal application is generally considered safe, as nitrous oxide causes minimal effects on respiration and hemodynamics, compared to other anaesthetic drugs [4]. N_2O is included in WHO List of essential medicines [5].

Recreational use of nitrous oxide comes from its ability to induce euphoria, relaxation and hallucinations upon inhalation (hence its colloquial name “laughing gas”) [6]. The outburst in abuse cases is directly connected to its availability, low cost and relaxed legal status nowadays. Main source of N_2O are commercial products, originally designed for food industry. Due to its overall low toxicity and extreme fat solubility it is approved as food additive E942 and is widely used as aerosol propellant in cooking sprays, e.g. whipped cream. Although the use of laughing gas for recreational purposes gained some popularity as early as 1799 (shortly after its discovery in 1772 by Joseph Priestley) and since then has been used sporadically for entertainment through XIX and XX century, recreational abuse of N_2O after 2000 grown drastically [6]. In recent years, the problem has increased to epidemic proportions, turning into a social issue for number of countries, such as China, Thailand, Vietnam, Australia and New Zealand [7-11]. According to European Union Drugs Agency (EUDA) reports, N_2O use has increased in some European countries since 2010 as well, and became a particular concern from around 2017-18 (Fig. 1), especially among young adults and adolescents [12]. In an attempt to handle the problem, most countries are introducing appropriate adjustments to local legislation – the process yet not finished.

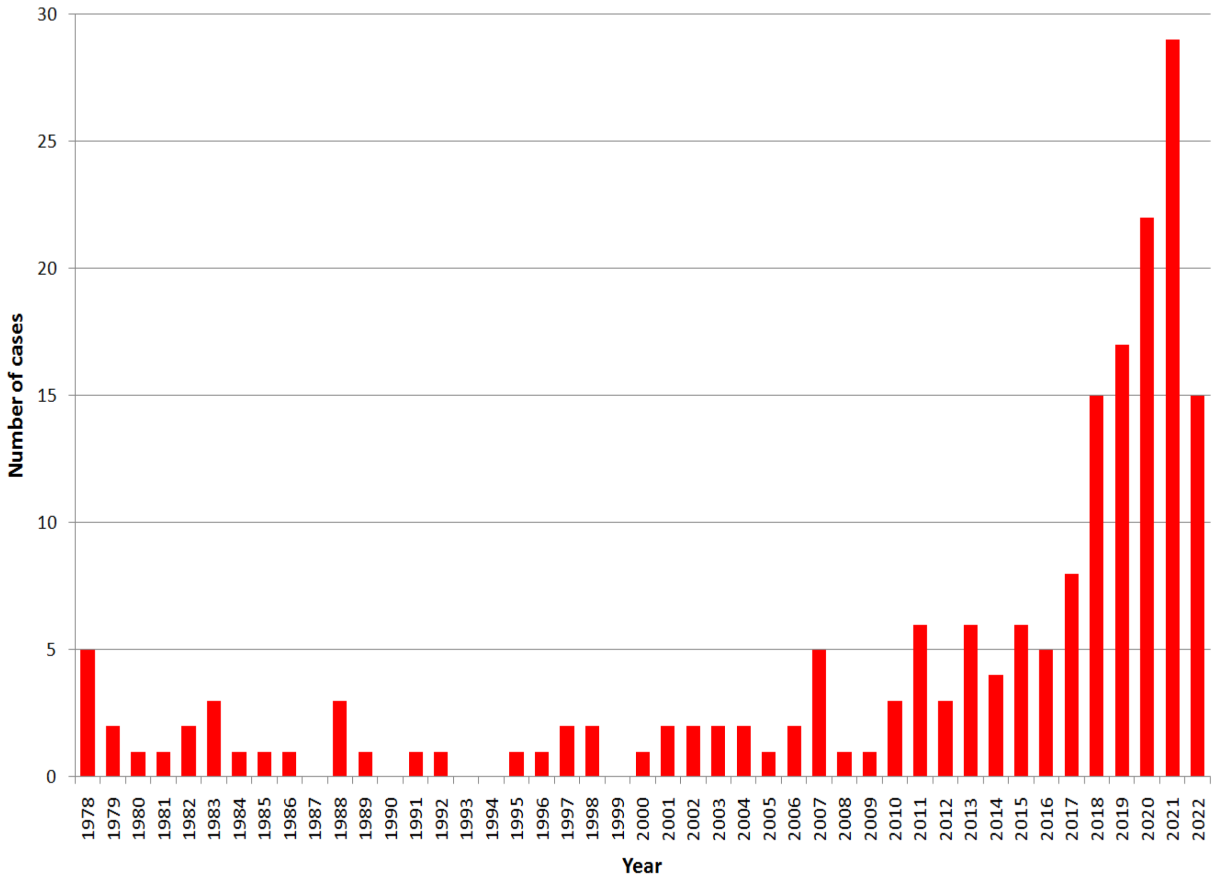

Fig. 1. Number of reported serious harms cases due to nitrous oxide abuse within Europe, PubMed database [12].

In small doses, administered by inhalation, nitrous oxide shows low acute toxicity. Some minor adverse effects (dizziness, light-headedness, disorientation, headache, tingling sensation, nausea, loss of coordination and fainting) may occur, although they are usually short-lived and fully reversible [12]. The gas is absorbed through the lungs and eliminated practically unchanged via respiration, as in humans less than 0.004% is metabolized [13]. The elimination half life is approximately 5 minutes [14]. The onset of action is rapid, effects start almost immediately, peak at about 10-30 seconds, and expire within 1-5 minutes. Isolated accidents occur rarely, mostly due to acute hypoxia (provoked by consumption of large quantities N_2O, e.g. 50 or more balloons in a single session, or release of the gas into an enclosed space without adequate ventilation, e.g. in the car), as well as frostbite (in case the gas is being used directly from pressurized container, as its temperature may fall down to minus 55°C in the course of adiabatic decompression) [12]. Need for medical treatment of an acute N_2O intoxication is uncommon.

The real danger of nitrous oxide poisoning lies in its chronic abuse. Even though the exact mechanism of harmful effects is yet not fully understood, a large volume of clinical data has been collected. Most reports unanimously testify that long-term abuse lead to serious neurological damage via chronic hypoxia, irreversible inactivation of vitamin B_{12}, and modulation of the N-methyl-D-aspartate (NMDA) glutamate receptor [4, 6, 12]. An early sign of neurological damage is manifestation of peripheral neuropathy: ataxia or paresthesia [15]. Other symptoms of vitamin B_{12} deficiency include sensory neuropathy, myelopathy and encephalopathy may occur, especially in cyanocobalamin compromised individuals [16]. If timely diagnosed, the impairment could be partially reversible, yet persistent sensory damage, and even permanent paralysis has been reported [17]. Serious psychiatric disorders, including long-term ones, may develop as well [18, 19].

Providing analytical proves of nitrous oxide recreational use for diagnostic or forensic purposes appears to be extremely challenging task. Classical methods of analytical toxicology screening are not directly applicable, as N_2O is fully eliminated from typical body fluids and exhaled within minutes after the consumption. There are some reports for employing gas-chromatography techniques for (mostly) post-mortem N_2O determination, however the lack of reproducibility has proven discouraging [20]. Instead, a combined profile for indirect biological markers – such as serum vitamin B_{12}, methylmalonic acid (MMA) and homocysteine (tHcy) levels – agreeably give the most clinical relevance [21].

The legal status of nitrous oxide in Bulgaria includes mandatory policies against spreading of the N_2O (Health law, since 2022), the latter declared punishable by law (Penal code, since 2024). Being a criminal offence, sales and distribution of N_2O are objects of prosecution, therefore laboratories of analytical toxicology are often asked to examine the physical evidences (such as cartridges, canisters and tanks), found at crime scenes, in order to identify the presence of N_2O in the content.

MATERIALS AND METHODS

Two 950 mL by volume and 1437 g by net weight canisters, containing unknown pressurized gas for identification (expected pressure 180 bar) were presented as physical evidences by prosecution authorities. Nitrous oxide anesthesiology grade was used as reference material.

GC-MS analysis was done on Agilent Technologies 7890B GC System & 5977A MSD module. Data acquisition and processing were controlled by Agilent MassHunter software package. Reference data from mass spectral library NIST version 2.0 g was used for comparison.

RESULTS AND DISCUSSION

Samples were collected directly in empty 2 mL air-tight screw-top borosilicate glass CG vials, displacing the existing air by the gas flow, regulated by valves provided with the canisters being examined. Additional efforts for evacuation of vials were proven not essential, as laboratory atmosphere contains no measureable quantities of N_2O, and, additionally, any traces of natural air, possibly remaining non-displaced in the GC vial, cannot introduce a noticeable interference in mass spectral data. GC analysis conditions are listed in Table 1. GC Column type was routinely used DB-1701 (30 m × 0.25 mm × 0.25 μm), which has no actual significance for the analysis, as simple gases usually show no specific adsorption, and, therefore, retention times are not useful for identification purposes.

Tabl. 1. GC-MS analysis conditions (SCAN mode).

Parameter	**Value**
Initial oven temp.	50°C
Oven ramp rate	none
Inlet mode	splitless
Carrier gas	He
Flow mode	constant flow
Flow rate	1.5 mL min^{-1}
Injection volume	2 μL
Total run time	5,0 min

The GC-MS identification of nitrous oxide was done by its characteristic fragmentation data at R_t = 1.33 min, mass spectrum (EI, 70 eV), m/z (Irel, ‰): **44*** (999), **30*** (377), **14** (169), **28** (142), **16** (62); qualifiers are marked by asterisk. The results obtained (Fig. 2) were in excellent agreement with both software NIST library and reference material MS spectrum data collected beforehand.

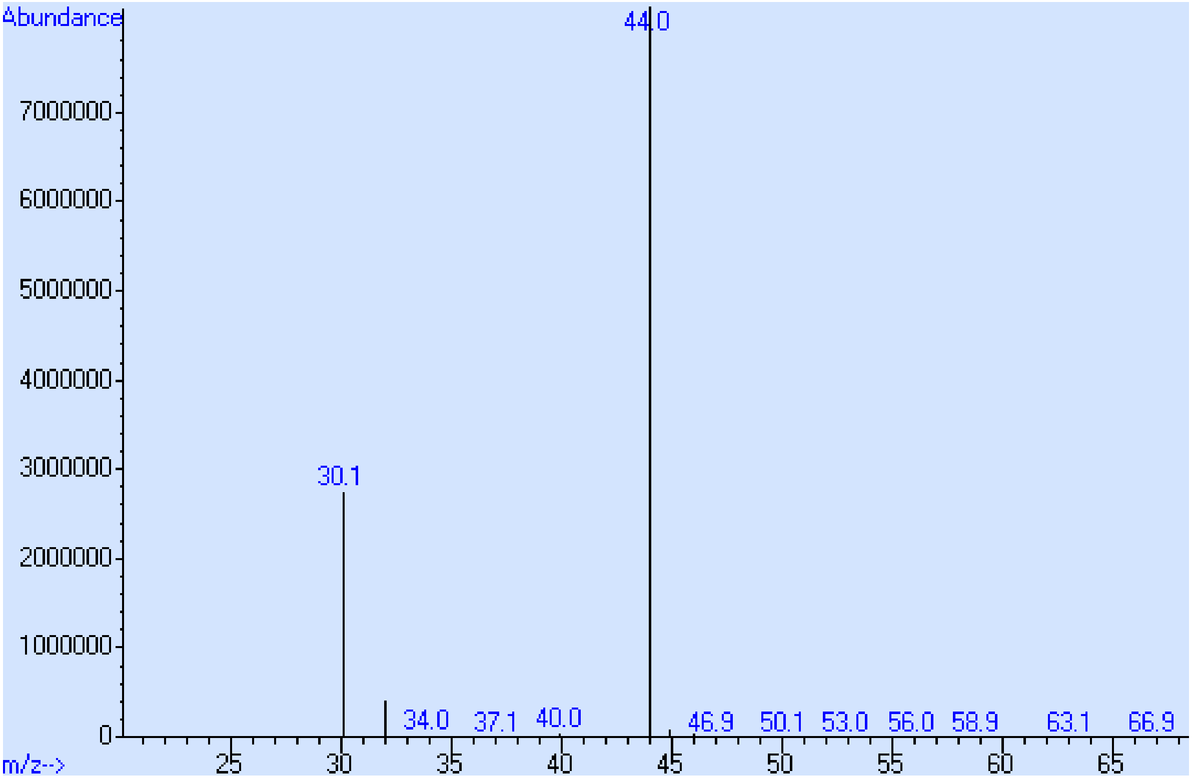

Fig. 2. Mass-spectrum of gas samples: identification of N_2O.

As it is shown on Fig. 2, the observed mass-spectrum of examined gas consists almost entirely of N_2O qualifiers, along with insignificant remains of air components molecular fragments. Carbon dioxide cannot interfere with the results obtained, as typical CO_2 qualifiers are not presenting with the sole exception of molecular ion m/z 44. Thereby, one could derive a conclusion, that presented canisters contain nitrous oxide as a main component.

In recent days, Bulgarian authorities have been discussing tightening the legal status of nitrous oxide and putting N_2O on the list of narcotic substances, completely prohibiting its application and distribution. If this happens, the application of the developed method will gain even greater importance in medico-legal practice.

CONCLUSION

Mass-spectral approach for reliable GC-MS identification of nitrous oxide content of physical evidences was applied. Analytical confirmation is achieved within 5 minutes, and no chemical reagents are needed. The method developed has been used in crime investigations as an instrument providing proofs needed in prosecution against illicit N_2O distribution.

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Address for correspondence:
G. Bonchev, Laboratory of Analytical Toxicology, Military Medical Academy, Naval Hospital – Varna, 3 Schristo Smirnenski Str., BG-9010, Varna, Bulgaria
e-mail: georgi.bontchev@gmail.com