Preprints.org

Article

Beta-Hydroxybutyric Acid as a Template for the X-ray Powder Diffraction Analysis of Gamma-Hydroxybutyric Acid

Domenica Marabello , Carlo Canepa , Alma Cioci , Paola Benzi [*]

Article

Beta-Hydroxybutyric Acid as a Template for the X-ray Powder Diffraction Analysis of Gamma-Hydroxybutyric Acid

Domenica Marabello [1,2], **Carlo Canepa** [3], **Alma Cioci** [4] **and Paola Benzi** [5,6,*]

[1] Dipartimento di Chimica, University of Torino, Via P. Giuria 7, 10125 Torino, Italy.
[2] Centre for Crystallography (CrisDi), University of Torino, Torino, Italy; domenica.marabello@unito.it
[3] Dipartimento di Chimica, University of Torino, Via P. Giuria 7, 10125 Torino, Italy; carlo.canepa@unito.it
[4] Dipartimento di Chimica, University of Torino, Via P. Giuria 7, 10125 Torino, Italy; alma.cioci@unito.it
[5] Dipartimento di Chimica, University of Torino, Via P. Giuria 7, 10125 Torino, Italy
[6] Centre for Crystallography (CrisDi), University of Torino, Torino, Italy
[*] Corresponding author: paola.benzi@unito.it

Abstract: In this paper we report the possibility of using the X-ray powder diffraction (XRPD) technique to detect the gamma-hydroxybutyric acid (GHB) as its sodium salt in different beverages but because it is not possible to freely buy them, beta-hydroxybutyric acid (BHB) and its sodium salt (NaBHB) were used as a model to fine tune an X-ray diffraction method for the qualitative analysis of the sodium salt of GHB. The method requires a small quantity of beverage, and an easy sample preparation, that consists only in the NaOH addition to the drink and a subsequent drying step. The dry residue obtained can be easily analyzed with XRPD using a single crystal X-ray diffractometer to exploit its high sensitivity that allows very fast patterns collection. Several beverages with different NaBHB:NaOH molar ratios were tested and the results show that NaBHB is detected in all drinks analyzed when NaBHB:NaOH molar ratio is 1:50 using characteristic peak at very low 2θ values that permits to detect its presence also in complex beverage matrices. Moreover, depending on the NaOH amount added, shifting and/or splitting of the characteristic peak of the NaBHB salt was observed, and the origin of this behavior was investigated.

Keywords: X-ray powder diffraction; non-destructive analysis; spiked beverages; γ-hydroxybutyric acid (GHB); β-hydroxybutyric acid (BHB); drink analysis

Remove this paragraph and start section numbering with 1. For any questions, please contact the editorial office of the journal or support@mdpi.com.

1. Introduction

Gamma-hydroxybutyric acid (GHB) is a short-chain fatty acid naturally present in all the mammals [1]. It acts as a precursor and metabolite of the inhibitory neuro-transmitter gamma-aminobutyric acid (GABA) and it is able to produce sedative, hypnotic, and anesthetic effects [2-3]. The sodium salt of GHB is registered as a therapeutic agent for the treatment of narcolepsy (Xyrem®) [4,5] and alcohol dependence to alleviate withdrawal symptoms [6,7]. Nevertheless, over the last few years due to its chemical-physical, organoleptic, pharmacodynamic and pharmacokinetic properties, it is used as recreational or abuse drug and its misuse has become a serious social problem, since it is often related to drug-facilitated crimes (DFC) as, for example, drug-facilitated sexual assault (DFSA) [8,9].

Drug-facilitated crimes are defined as crimes such as robbery, money extortion, cheats, battery, sexual assault (DFSA) when committed while the victim is under the influence of a drug [8-13] and hence with impaired behaviour, perception, or decision-making capacity.

Two main different circumstances in DFC cases occurs: 1) when the perpetrator takes advantage of someone's voluntary use of alcohol or other drugs (proactive (DFC); 2) when the perpetrator intentionally forces someone to consume alcohol or other drugs with or without their knowledge (opportunistic DFC).

In this scenario, GHB can be voluntary ingested, for recreational purposes (for example, in party contest or discotheque). In fact, at low dose it induces a state of euphoria [14,15], disinhibition and relaxation. Nevertheless, more dramatic effects can be occurring due to the individual sensibility to the drug [14,16] and because the users are often unaware of the dose they are consuming considering that the actual quantity of GHB in drugs illegally bought on the street or online is not known.

On the other hands GHB is well suited to being used for criminogenic purposes, such DFC [9-12] since it is colorless, odorless and soluble in both alcoholic and non-alcoholic beverages, easily masking its flavor and it is rapidly absorbed by the body. Moreover, if the dose is increased, a state of sedation and stunning takes over, which allows the persecutor to prevail and overcome the resistance of the victim. Finally, it causes a state of retrograde amnesia, which prevents the reconstruction of the violence suffered [15,17].

For this reason, in many countries GHB is classified as a controlled substance.

Many papers and reviews [8,9,11,18-21] were published reporting about the psychoactive substances used in DFC (and particularly DFSA) and even if GHB is not one of the most frequently detected drugs, the possible risk of GHB in this regard must not be neglected also considering that, the GHB involvement in this type of crimes can be largely underestimated.

In fact, the most common matrices used for forensic purposes are biological (blood, saliva, urine, hair and others) [22] and GHB can be detected with different analytical techniques [22,23] based on liquid or gas chromatography coupled with mass spectrometry [24-30], nuclear magnetic resonance spectroscopy [31-33,35], IR and Raman spectroscopy [34-41]. Colorimetric, fluorimetric and sensing techniques were also employed [42-45]. Nevertheless, GHB has relatively short half-life and is rapidly metabolized and eliminated from the body and it is not detectable in blood after 2-8 hours and in urine after 8-12 hours [46-48]. Moreover, there is often a delay of hours or even days between reporting a possible drug facilitated crime, which can compromise the possibility to determinate the exogenous GHB in this kind of samples.

For these reasons, the true incidence of GHB use in DFC may be higher than previously recognize. Therefore, after filing a police report of proactive or opportunistic GHB-DFC involving suspected spiked drink, it becomes a crucial point the careful evaluation of the matrices to collect and analyse and, as part of the investigation, it is highly important to identify the GHB in the suspected beverage if available [49].

For this purpose different methods were reported [17,29,30,50-55] that require time consuming sample preparations, involving, for example, extraction and/or derivatization procedures.

Moreover, a complicating factor is the interconversion of GHB to the lactone form (gamma-butyrolactone, GBL), that is pH dependent: the more is the acidic condition, the more the GHB converts to GBL, while under strongly alkaline conditions the GBL is completely converted to the GHB form [36,56].

Among the analytical techniques for forensic purposes, X-ray powder diffraction (XRPD) is an attractive method for crystalline and semi-crystalline materials characterization [57-61] and it will be extremely promising for the analysis of new and more classical street samples of psychoactive substances [62-64]. In fact, XRPD allows not-destructive analyses and to distinguish between substances even with similar chemical structures, that are fundamental requirements in the forensic analysis of psychoactive substances.

The aim of this work is to propose a new XRPD method for the GHB analysis in beverages using a diffractometer for single crystal diffraction technique.

In fact, the most diffused XRPD diffractometers, equipped with point detectors, require grinding of samples to assure the casual orientation of crystals and obtain a good reproducibility of the measurements. Instead, the faster CCD detectors, normally mounted on diffractometers for single crystal diffraction technique, allow to calculate peak intensities integrating over all the diffracted

circles, and hence to collect reproducible patterns also with oriented or not ground samples. Moreover, these instruments are characterized by high sensitivity of CCD detectors and intense high-focalized X-ray beams, allowing the collection of good quality powder diffraction patterns also with a very small quantity of materials. This is particularly interesting for not-destructive analysis of GHB in dried residues of adulterated beverages for DFC.

GHB and its lactone form are present in the list of substances subjected to D.P.R. n. 309/90 of the Italian Ministry of Health [65,66] and their use and sale are strictly regulated by law, and thus it is not possible to freely buy them. Consequently, in this paper the β-hydroxybutyric acid (BHB) and its sodium salt (NaBHB), were used as models to fine tune a method for a qualitative analysis in different beverages using X-ray diffraction. In fact, BHB and NaBHB can be considered structural analogues of GHB and NaGHB respectively, and it is reasonable to expect they have the same chemical behavior.

The method developed requires a very easy sample preparation and it allows to perform the analysis rapidly. Moreover, if used for GHB (NaGHB) analysis, it prevents the problems connected to with GHB– GBL interconversion [36,56].

2. Results and Discussion

Figure 1 compares the two powder patterns of NaBHB standard, obtained with the two procedures described in the *Standard preparation* section. The pattern of NaOH recrystallized from aqueous solution is also shown.
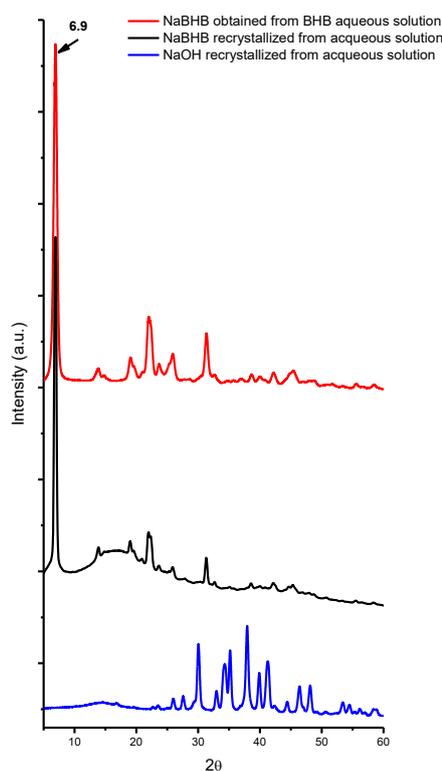

Figure 1. XRD pattern of NaBHB obtained from BHB+NaOH in aqueous solution, NaBHB and NaOH recrystallized from aqueous solutions. The patterns have been translated on the ordinate axis and normalized with respect to the higher signal of NaBHB.

Taking into account that the very large band, visible in the background of the patterns in the 2θ range 8.5÷20°, is attributable to added paraffin, the same XRPD pattern is obtained for the two standards. The NaBHB pattern is characterized by a high and sharp peak at 2θ value 6.9°, while at higher 2θ values less intense and more superimposed peaks are present.

For NaGHB a similar pattern was observed [67], with a high and sharp peak at the 2θ value of 6.16°, and this fact supports the use of BHB and NaBHB as models to tune a method for XRPD analysis of GHB and NaGHB.

Series A samples. To verify the possibility to detect the NaBHB with the XRPD for the qualitative analysis in the beverages residue, a preliminary analysis on three samples, coke, scotch, and red wine, (non-alcoholic and with high and low alcohol content, respectively, see Table 2) spiked with a considerable amount of BHB (20% v/v) and with 1:1 BHB:NaOH molar ratio, was performed using the procedure described in the Materials and Methods section.

The XRPD patterns of the dry residues are reported in Figure 2. For all samples, the low angle peak of NaBHB is clearly visible, indicating that BHB (in the NaBHB form) can be easily detected. The same results are obtained if the beverages are spiked with NaBHB (Series A2) as shown in Figure A.A2 of the Appendix A.
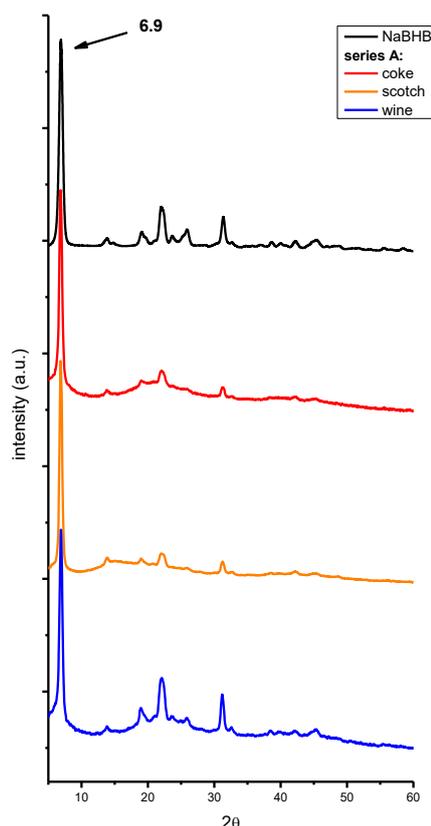

Figure 2. XRPD patterns of the solids obtained from different beverages spiked with 20% v/v of BHB and 1:1 BHB:NaOH ratio (series A). The pattern of NaBHB obtained from the BHB aqueous solution is also reported for comparison. The patterns have been translated on the ordinate axis and normalized with respect to the higher signal of NaBHB.

Series B1 samples. For illicit purposes, the quantities usually added to the beverages range from 1 to 5 grams of NaGHB or from 2 to 10 mL of pure GHB, to a volume of 100 or 200 mL of drink [14-17,29,50]. Therefore, a second series of samples (series B1) of several common beverages (coke, beer, rum, scotch, and wine) was analyzed. These samples were spiked with a lower BHB percentage (1% v/v) and 1:2 stoichiometric BHB:NaOH molar ratio to favour the formation of the sodium salt. The results of the X-ray analyses of the dry residues, reported in Figure 3, evidence signals attributable to NaBHB only for scotch and rum. Analogous results (shown in Figure A.B2 of the Appendix A) were obtained with beverages directly spiked with 1% w/v of NaBHB (series B2 samples).
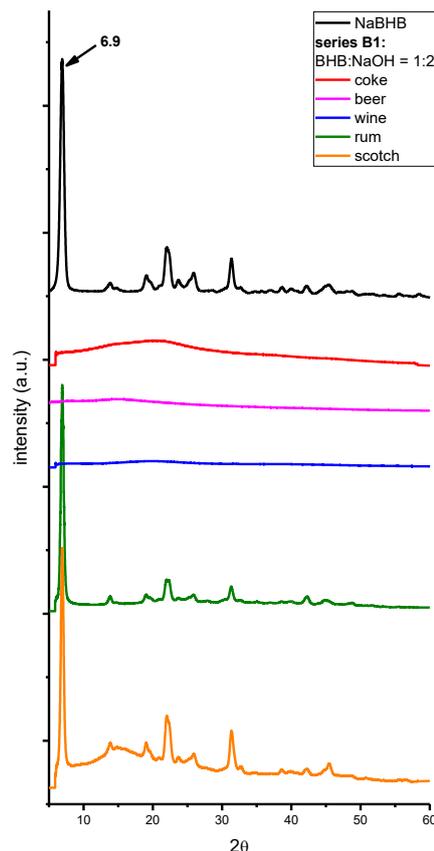
Figure 3. XRPD patterns of the residues of some beverages, spiked with 1% v/v of BHB and 1:2 BHB:NaOH ratio (series B1). The pattern of the standard NaBHB obtained from the BHB aqueous solution is also reported for comparison. The patterns have been translated on the ordinate axis and normalized with respect to the higher signal of NaBHB.

Series B3 samples. Moreover, samples of the same beverages without BHB but added with the same NaOH content of series B samples were prepared to verify if naturally occurring GHB [30,68,69] or other chemical substances present in the beverage matrix can lead to the formation of crystalline compounds after the drying step. The XRPD patterns reported in Figures A.B3 of the Appendix A, do not evidence the presence of peaks due to crystalline species, except for the rum and scotch samples, that show only weak signals attributable to NaOH.

To explain the absence of the NaBHB signal for coke, beer and wine of Serie B1 samples, two hypotheses can be considered. The first hypothesis is a low sensibility of the analytical method. In fact, it must be considered that (i) after the drying step only a small part of the residue is used since a very small quantity of sample is needed for X-ray diffraction analysis; (ii) due to the composition of the beverages, different amounts of dry residue (g/ml) are obtained and hence the NaBHB percentage with respect to the total weight of the residues varies a lot from one beverage to another.

Table 1 reports the amount of dry residue obtained for the not spiked beverages, and the calculated percentage of NaBHB with respect to the total weight of the residues for the spiked samples with 20% v/v of BHB and 1:1 BHB:NaOH molar ratio; the percentage for the spiked beverages with 1% v/v of BHB and 1:2 or 1:50 BHB:NaOH molar ratios are also reported.

Table 1. Tested beverages, dry residue (g/ml) of not spiked beverages and NaBHB weight percentage with respect to the total weight of the residues obtained after the drying step of the spiked samples with 1% and 20% v/v of BHB and different BHB:NaOH molar ratios.

beverage	dry residue (g/ml)	NaBHB weight percentage (w/w)		
	(not spiked)	BHB 1% v/v BHB:NaOH 1:2	BHB 20% v/v BHB:NaOH 1:1	BHB 1% v/v BHB:NaOH 1:50

| coke	0.099	11.2	72.3	4.1
beer	0.037	23.9		5.2
red wine	0.025	30.7	91.2	5.4
rum	0.002	67.8		5.5
scotch	0.001	71.6	96.3	5.8

As expected, the unspiked different beverages have different dry weight and therefore when the BHB is added the percentage of NaBHB in the dry residue can vary widely.

The series A samples were added with a high amount of BHB and hence the percentage of NaBHB in dry residue is very high (from 72.3 to 96.3%) allowing NaBHB to be easily detected. Moreover, from the data in Table 1, it is clear that, even for rum and scotch of series B the percentage of NaBHB is high (higher than 65%). On the contrary, for coke, beer, and red wine it is less than 31%. As reported above, NaBHB was detected in the rum and scotch samples, but not in the coke, beer, and red wine. Therefore, it could be hypothesized that the low NaBHB weight percentage in the analyzed dried residues can be responsible for the negative results obtained for some of the spiked beverages with 1% of BHB.

The second hypothesis is that these negative results are due to secondary reactions of NaOH with other substances in the beverages matrix that prevent the reaction between the BHB and the NaOH leading to NaBHB. In fact, in the beverages different acidic substances (as carbonic acid in fizzy drinks, phosphoric acid in coke or carboxylic acids in alcoholic drinks) [70,71] are present and can subtract the added NaOH preventing the reaction between the BHB and the NaOH leading to NaBHB. Moreover, BHB, being a weak acid, is in equilibrium with its deprotonated form and the presence of acidic substances, favours the BHB form.

In the beverages spiked with 1% of BHB and 1:2 BHB:NaOH molar ratio, the amount of NaOH is small, and it could be employed to the secondary reactions, leaving too small amount of NaOH for the salification of the BHB. The fact that in the XRPD patterns of the series B samples the peaks of NaOH are not visible supports this hypothesis, since NaOH is in excess with respect to the BHB amount. On the contrary, in the beverages spiked with 20% of BHB, the NaOH amount is higher, being 1:1 the BHB:NaOH molar ratio, and it is reasonable to hypothesize that even after matrix reactions part of NaOH is still available for the salification reaction.

Serie C, D, E, F samples. To explore the second hypothesis, different series of samples with 1% of BHB and BHB:NaOH molar ratios 1:5, 1:10, 1:20, 1:50 (series C, D, E, F, respectively) were prepared. The XRPD patterns are shown in Figure 4 (C-F). As for series B samples, only the rum and scotch samples of series C (BHB:NaOH molar ratio 1:5) show the NaBHB signals. Moreover, it must be noted that if the NaOH content is further increased (from series C to F), the XRPD patterns are complicated by the appearance of the NaOH signals and when the BHB:NaOH molar ratio is 1:50 it is clear that the peaks over 2θ values of 20° are attributable to NaOH.
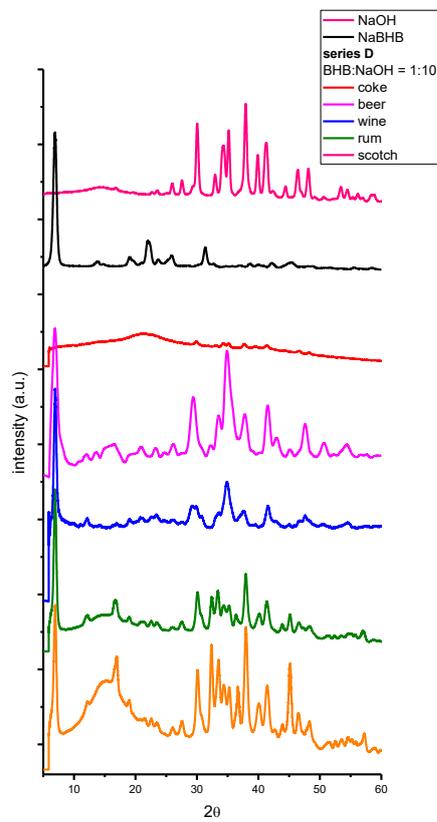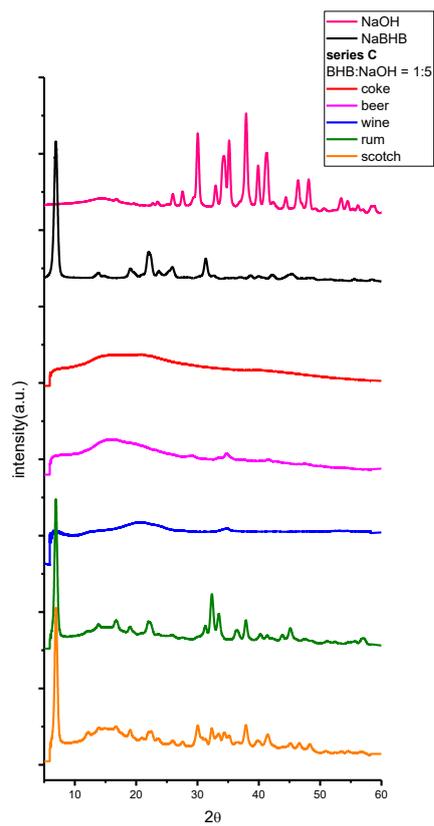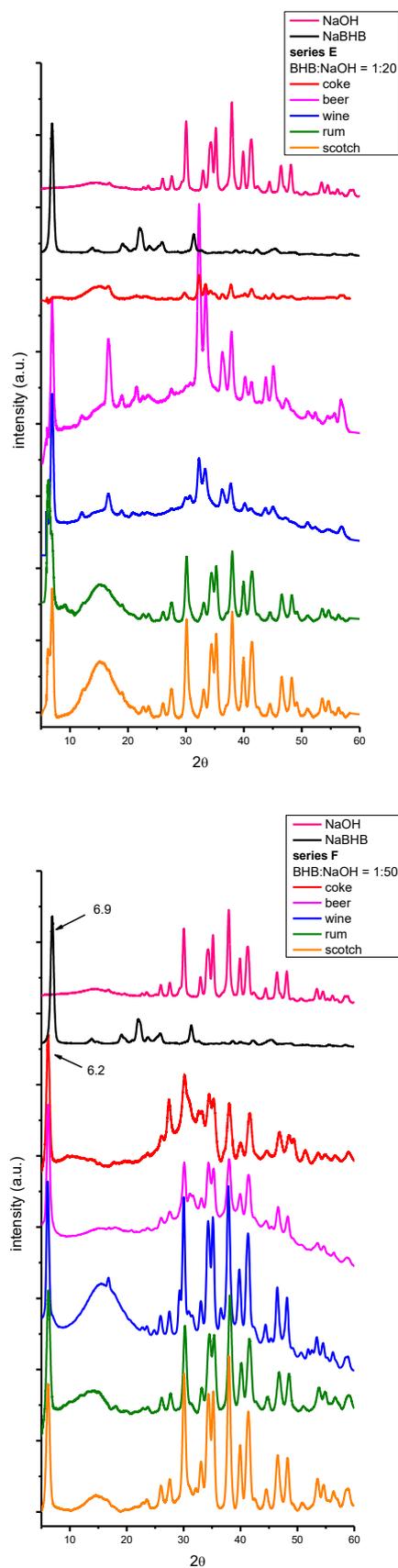
Figure 4. (C-F). X-ray patterns of the solid obtained from some beverages spiked with 1% v/v of BHB and BHB:NaOH ratio 1:5 (C), 1:10 (D), 1:20 (E), 1:50 (F). The patterns of NaBHB obtained from the BHB aqueous solution and of recrystallized NaOH are also shown. The patterns have been translated on the ordinate axis and normalized with respect to the higher signal of NaBHB.

Nevertheless, the most intense peak at low 2θ value, typical of NaBHB, is always clearly visible if the salt is present. Again, the large band in the 2θ range between 8.5° e 20° visible in the patterns of some samples of Figures 4 is attributable to the paraffin used to compact the powders.

Moreover, as described in the Materials and Methods section, the samples are not ground before the analysis and hence the distribution of NaBHB and NaOH in the solid residue are not uniform. For this reason, the ratios between the higher peak of NaBHB and those of NaOH do not follow a regular trend with increasing of the NaOH amount.

Furthermore, Figure 4 evidences that as the NaOH content was increased, the low angle peak of NaBHB becomes evident, even in the XRPD patterns of beer and wine samples of series D and E and in all the samples of the series F.   This finding supports the second of the previously reported hypotheses and contradicts the first: in fact, if the NaOH content increases, the NaBHB weight percentage with respect the total weight of dry residue decreases. For example, for the scotch and coke samples, the NaBHB weight percentage varies from 71.6% to 5.8% and from 11.2% to 4.1% respectively, if the BHB:NaOH molar ratio varies from 1:2 to 1:50 (see Table 1).

Another interesting observation can be made comparing the position of the highest peak of NaBHB with the increasing of NaOH amount: for BHB:NaOH molar ratio up to 1:10 the peak is positioned at 6.9°, while when BHB:NaOH molar ratio is 1:50 it is at ca. 6.2°. Moreover, when the BHB:NaOH molar ratio is 1:20, for rum and scotch samples, the higher peak is splitted into two signals at 6.2° and 6.9°. In Figure 5 are reported the XRPD patterns of scotch samples at different BHB:NaOH molar ratios, as example, to evidence this behaviour. In the inset is evidenced the zone between 5° and 20° 2θ values. (The XRPD patterns of rum samples are shown in Figure A5 of the Appendix A).
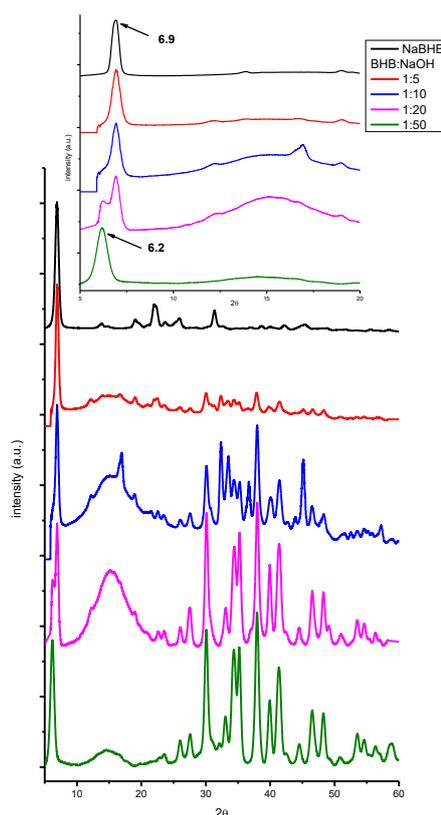

Figure 5. X-ray patterns of dry residues from the scotch samples spiked with 1% v/v of BHB and BHB:NaOH ratios 1:5, 1:10, 1:20, 1:50. In the inset is evidenced the zone of 2θ values between 5 and 20 degree. The patterns have been translated on the ordinate axis and normalized with respect to the higher signal of NaBHB.

Series G samples. To understand this results, a series of water solutions with 1% of BHB and different BHB:NaOH molar ratio (from 1:2 to 1:50) (series G) was prepared. The results of XRPD

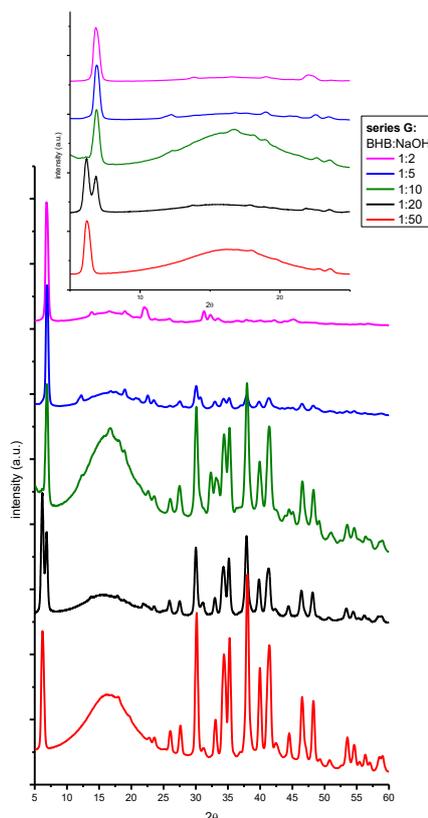analyses are shown in Figure 6. In the inset is evidenced the zone of 2θ values between 5 and 20 degree.

Figure 6. X-ray patterns of the dry residues obtained from water solutions spiked with 1% v/v of BHB and BHB:NaOH ratio from 1:2 to 1:50. In the inset is evidenced the zone of 2θ values between 5 and 20 degree. The patterns have been translated on the ordinate axis and normalized with respect to the higher signal of NaBHB.

Even in this case, in solutions with 1:2, 1:5, 1:10 BHB:NaOH molar ratios the highest peak of NaBHB is found at 2θ=6.9°. Furthermore, in the solution with 1:20 molar ratio the signal at 6.2° also appears, while in the one with molar ratio 1:50 the peak at 6.9° disappears, leaving only the signal at 6.2°. This behavior suggests the possibility of the formation of two different polymorph of NaBHB, with a similar structure, depending on the NaOH concentration: the transition from one to the other is observed increasing the NaOH content.

Series H samples. Considering the above results, the XRPD technique was used to analyse different beverage samples (listed in Table 2) (series H) spiked with 1% of NaBHB (w/v) and NaBHB:NaOH molar ratio 1:50. The results are shown in Figure 7 A, B and C.
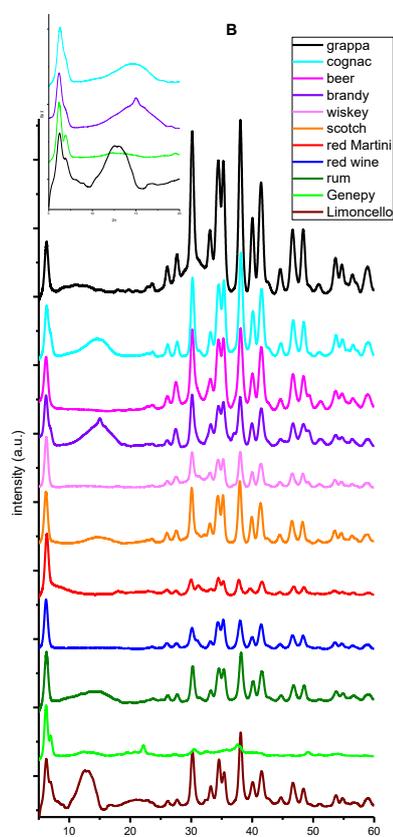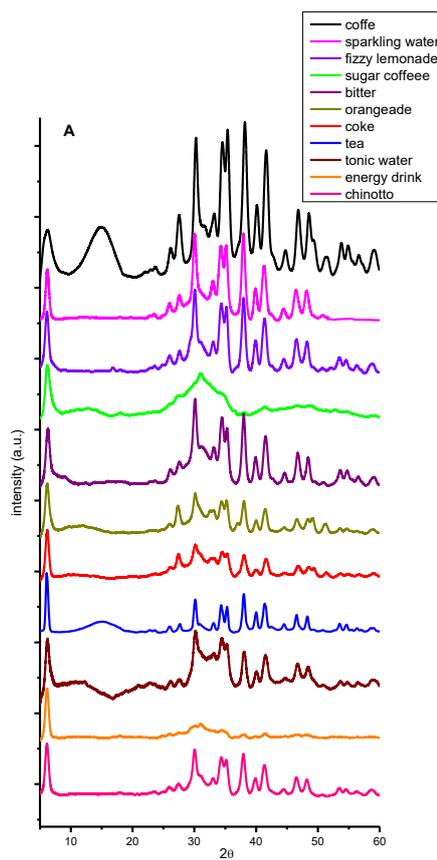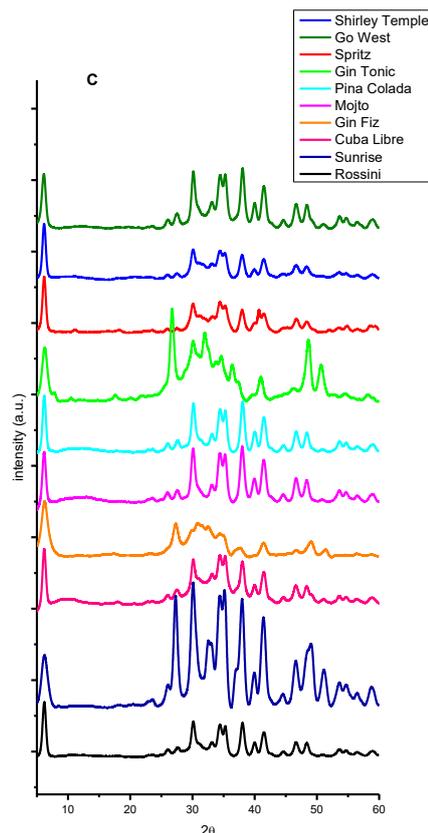
Figure 7. XRPD patterns of some analcoholic (A), alcoholic (B) and cocktails (C). The patterns have been translated on the ordinate axis and normalized with respect to the higher signal of NaBHB.

As can be observed in the powders patterns of figure 7, the characteristic signal at 2θ value 6.2° is clearly visible in all the beverages considered. Thus, it can be concluded, that under these experimental conditions, the presence of NaBHB can be detected in all the drinks analysed. Nevertheless, it must be noted that for some samples (cognac, brandy, Genepy and Limoncello) also the signal at 2θ value 6.9° is present (as evidenced in the inset of Figure 7 B), indicating that both the two supposed polymorphs are contemporary present.

3. Materials and Methods

3.1. Materials

Sodium Hydroxide, β-Hydroxybutyric Acid (BHB) as 95% (w/w) Solution with Density of 1.126 g/mL, and Sodium β-Hydroxybutyrate (NaBHB), were Purchased from Sigma-Aldrich.

The alcoholic and non-alcoholic beverages were purchased from some local supermarket and cocktail bars.

3.2. Standard Preparation

In order to perform a diffraction analysis, the sample must be in a solid and crystalline form and thus generally X-ray diffraction is not a suitable technique to analyze drugs dissolved in beverages. BHB, as its analogue GHB, is a molecular liquid, but it can be easily turned to the solid state adding a base, such for example NaOH, to form the NaBHB salt. Thus, to compare the XRPD patterns of the samples, first of all the XRPD pattern of the standard NaBHB salt, that was not available in literature, is needed. The NaBHB salt was synthetized starting from a BHB aqueous solution, following the same procedure adopted by Gorecho *et al.* [72] to obtain the NaGHB from the GHB: an equimolar quantity of BHB is added to a 5.0 M solution of NaOH, and the solution is dried at 50 °C, until a precipitate is formed. Finally, the precipitate is filtered and washed with acetone. After a rapid drying step, the diffraction pattern was collected. Since GHB is introduced by the perpetrators into the adulterated

beverages also directly in the salt form, the diffraction pattern of purchased NaBHB recrystallized from its aqueous solution was also collected.

3.3. Sample Preparation

To perform the XRPD analysis on the liquid beverages, a procedure is required to turn the samples into the solid form. In this work an easy sample preparation, that involves a first step of addition of a base (NaOH) to the drink, in order to neutralize the BHB molecule, and a second drying step of the beverage, to obtain the drug in a solid crystalline form (NaBHB salt) inside the dried residue of the beverage was developed. Considering that during a law enforcement investigation about a suspected spiked drink the amount of beverage collected can be very low because it consists of the residue after drinking, for each beverage an amount of only 50 microlitres (μL) was used.

The tested beverages are listed in Table 2. The recipes of the cocktails are reported in the Appendix A.

Table 2. Tested beverages. Note: ABV is the Alcohol By Volume (%) content.

analcoholic	alcoholic	cocktail
coffee (Nespresso)	white wine schnapps (Candolini 40.0 ABV)	Shirley Temple
sparkling water (Sparea - Pontevecchio)	cognac (Couvoisier 40.0 ABV)	Go West
fizzy lemonade (Lemonsoda- Royal Unibrew)	beer (Carlsberg 5.0 ABV)	Aperol Spritz
sugary coffee (Nespresso)	brandy (Vecchia Romagna 37.2 ABV)	Gin Tonic
bitter (Crodino - Campari Group)	whiskey (Jack Daniel's 40.0 ABV)	Piña Colada
orangeade (Fanta - Coca-Cola HBC Italia)	schotch (J&B 40.0 ABV)	Mojto
coke (Coca Cola - Coca-Cola HBC Italia)	red Martini (14.4 ABV)	Gin Fiz
tea (Lipton)	red wine (Dolcetto 13.5 ABV)	Cuba libre
tonic water (Schweppes- San Benedetto)	rum (Havana Club 40,0 ABV)	Sunrise
energy drink (Red Bull – Rauch)	genepy (Alpe Herbetet 38,0 ABV)	Rossini cocktail
chinotto (Lurisia)	limoncello (Limoncè 25,0 ABV)	

The solutions of spiked beverages were prepared adding the appropriate quantity of drug to 50 μL of each beverage, and adding NaOH to induce the formation of the BHB sodium salt. After manual stirring, the solutions were dried and the residues analyzed with XRPD. Each beverage was tested three times. The samples were not ground before XRPD analysis. To avoid moisture absorption, after drying, the samples were stored in oven at 60 °C or in capped vials.

Samples Series. Solutions containing 20% and 1% (v/v) of BHB or 1% (w/v) of NaBHB were used and different BHB:NaOH (or NaBHB:NaOH) molar ratios were tested (from 1:1 to 1:50).

Serie A samples. The first series of samples consists of coke, scotch, and red wine, containing 20% v/v of BHB. To promote the transformation of BHB in its sodium salt, sodium hydroxide was added to have 1:1 stoichiometric BHB:NaOH molar ratio, before the drying step.

Series A2 samples. Coke, scotch, and red wine spiked with 20% w/v of NaBHB and added with NaOH to have 1:1 stoichiometric NaBHB:NaOH molar ratio.

Serie B1 samples. Coke, beer, rum, scotch, and red wine containing 1% v/v of BHB and added with NaOH to have 1:2, stoichiometric BHB:NaOH molar ratio.

Series B2 samples. Coke, beer, rum, scotch, and red wine containing 1% w/v of NaBHB and added with NaOH to have 1:2, stoichiometric NaBHB:NaOH molar ratio.

Series B3 samples. Coke, beer, rum, scotch, and red wine without BHB but added with the same NaOH content of series B samples.

Serie C, D, E, F samples. Coke, beer, rum, scotch, and red wine containing 1% v/v of BHB and added with NaOH to have 1:5, 1:10, 1:20 and 1:50 stoichiometric BHB:NaOH molar ratio, respectively.

Series G samples. Water solutions with 1% of BHB v/v and added with NaOH to have 1:2, 1:5, 1:10, 1:20 and 1:50 stoichiometric BHB:NaOH molar ratio, respectively.

Series H samples. Beverage samples listed in Table 2 spiked with 1% of NaBHB (w/v) and added with NaOH to have NaBHB:NaOH molar ratio 1:50.

3.4. X-ray Powder Diffraction (XRPD).

Due to the low amount of samples analyzed, a very sensitive XRPD instrument is needed, and for this reason, an instrument normally used for single crystal X-ray diffraction was chosen. In fact, this instrument is equipped with a very sensitive area detector that furthermore allows to collect reproducible XRPD patterns, also with unground samples. In the case of beverages, the solid residue obtained is often hygroscopic and too small to allow the grinding, and thus the area detector is a fundamental requirement to obtain reproducible XRPD patterns.

The X-ray Powder Diffraction patterns (XRPD) were collected at room temperature using the Atlas S2 Rigaku-Oxford Diffraction Gemini R-Ultra diffractometer, equipped with mirror monochromatized Cu-Kα (1.5418 Å) radiation. A little portion of dried powdered sample is compacted and modelled as a ball of ca. 0.45 mm diameter (less than the diameter of the X-ray beam). In some cases, with powders difficult to compact, the sample is compacted with a little drop of paraffin oil (non-drying immersion oil for microscopy, type B, code 1248, Cargille Laboratories). The ball is placed on the tip of a glass capillary, which is mounted on the goniometer head of the instrument. Each powder pattern was collected by rotating the sample of 60 degrees, with an exposure time of 60 seconds.

4. Conclusions

In this paper, a XRPD technique to detect the presence of BHB and/or NaBHB in a range of nonalcoholic and alcoholic beverages was developed. NaBHB was detected in all drinks analyzed with NaBHB:NaOH molar ratio 1:50. For different NaBHB:NaOH ratios values, the typical low angle NaBHB peak was not detected in all the samples, probably due to the NaOH reaction with the sample's matrix, that prevent the formation of the NaBHB salt. This hypothesis is supported by the fact that the beverages with higher weight of dry residues need the higher NaOH amount to obtain a positive result.

It must be also noted that the characteristic low angle peak of the NaBHB salt is found at 2θ=6.9° for samples with 1:2 NaBHB:NaOH molar ratio, shifts to ~ 6.2° for samples with 1:50 NaBHB:NaOH molar ratio, and splits in both positions for intermediate values of NaBHB:NaOH molar ratios. This behavior suggests that at higher NaOH concentration a different polymorph of NaBHB is formed, with a similar crystal structure.

The method developed in this work has the great advantage of needing a very small amount of beverages (only 50 μL) and an easy samples preparation: only the NaOH addition to the drink and a drying step are required.

Furthermore, employing a highly sensitive instrument, as is a single crystal diffractometer, the grinding of the samples is not required and the collection of the XRPD patterns is very fast, allowing to obtain the result of the analysis in a very short time, that is of fundamental importance during a forensic investigation.

Since BHB is chemically similar to GHB, a used drug for drug-facilitated crimes, it is presumable that the results obtained in this work can be transferred to the analysis of GHB in the same beverages. In this case as additional advantage with respect to previous methods of analysis, it allows to avoid the GHB – GBL interconversion [36,56], since a strongly basic pH is used for the preparation of the dry crystalline residue.

Author Contributions: Conceptualization, Domenica Marabello and Paola Benzi; Data curation, Domenica Marabello, Alma Cioci and Paola Benzi; Formal analysis, Domenica Marabello, Carlo Canepa, Alma Cioci and Paola Benzi; Funding acquisition, Domenica Marabello; Investigation, Domenica Marabello and Alma Cioci;

15

Appendix A.

Cocktails recipes.

Shirley Temple
20 ml grenadine
100 ml Ginger Ale
1 lemon slice
ice cubes

Go West
30 ml Limoncello
30 ml white wine
15 ml Hazelnut Irish Cream
15 ml simple syrup
15 ml lemon juice

Aperol Spritz
60 ml Prosecco
1 splash club soda
44 ml Aperol
1 slice orange
ice cubes

Gin Tonic
50 ml gin
150 ml tonic water
1 lemon
ice cubes

Pina colada
50 ml White Rum
    30 ml Coconut Cream
    50 ml Fresh Pineapple Juice
ice cubes

Mojito
10 fresh mint leaves
44 ml white rum
½ medium lime
2 tablespoons white sugar
120 ml club soda
ice cubes

Gin Fizz
45 ml Gin
    30 ml Fresh Lemon Juice
    10 ml Simple Syrup
    Splash of Soda Water
ice cubes

Cuba libre

50 ml White Rum
     120 ml Coca Cola
     10 ml Fresh Lime Juice
ice cubes

Sunrise
90 ml orange juice
60 ml sparkling water
7 ml grenadine
ice cubes

Rossini
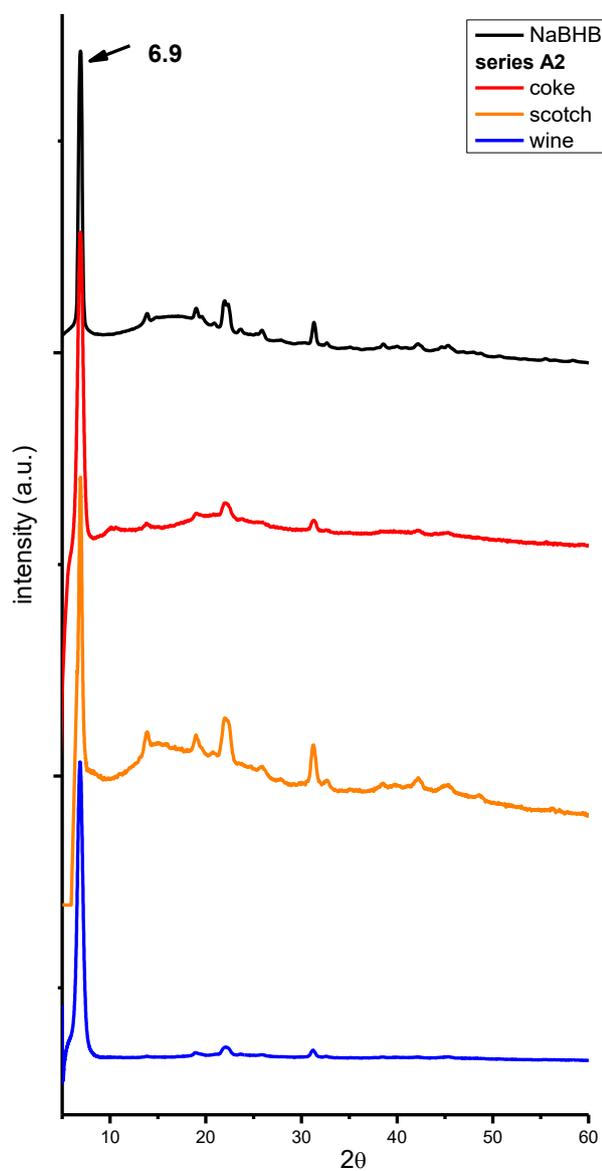50 g strawberries
10 g sugar
100 ml prosecco

Figure A2. XRPD patterns of the solids obtained *from different beverages spiked with 20% v/v of NaBHB* and 1:1 NaBHB:NaOH molar ratio. The pattern of NaBHB recrystallized from aqueous solution is also reported for comparison.

Figure 2. XRPD patterns of the residues *of some beverages, spiked with 1% v/v of NaBHB* and 1:2 NaBHB:NaOH molar ratio. The pattern of NaBHB recrystallized from aqueo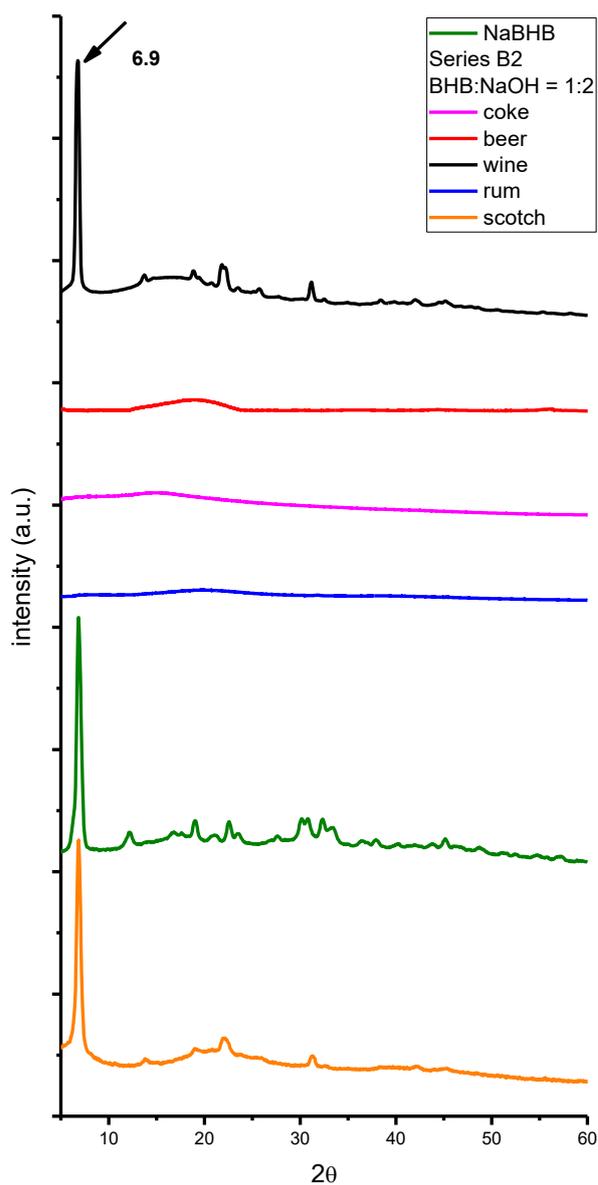us solution is also reported for comparison. The patterns have been translated on the ordinate axis and normalized with respect to the higher signal of NaBHB.
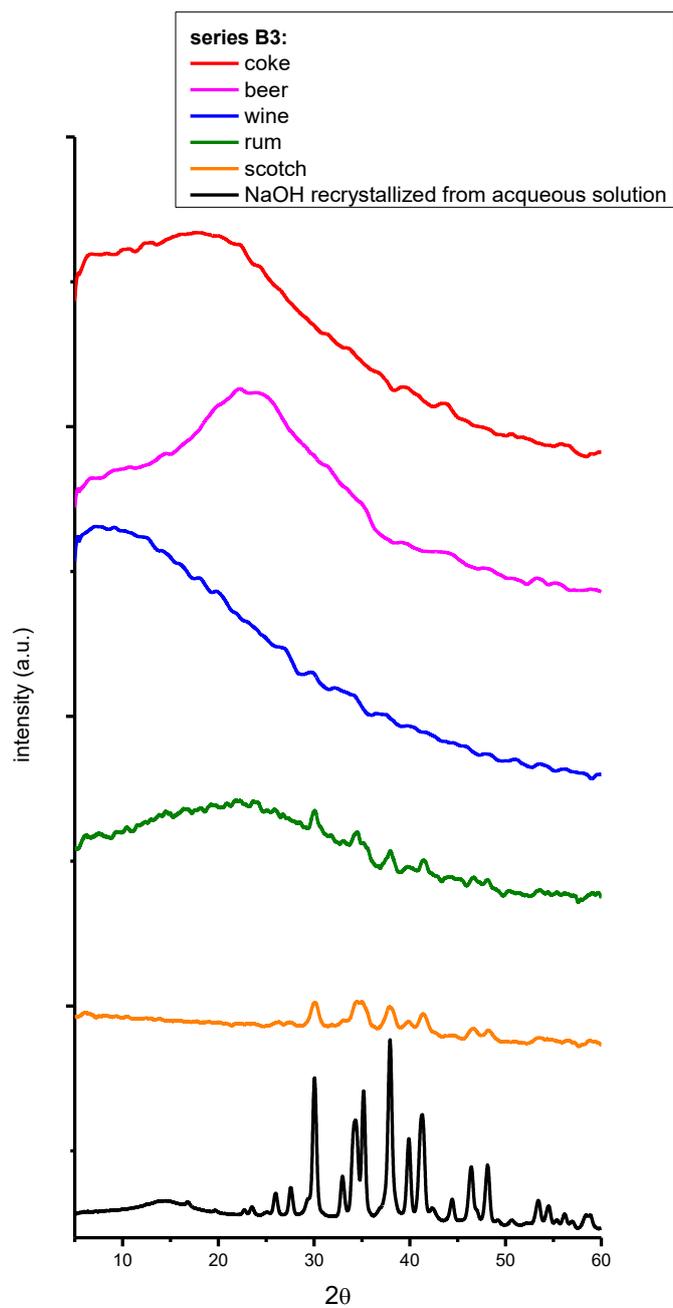
Figure 3. XRPD patterns of the residues of *some beverages without BHB but added with the same NaOH content of series B samples.* The pattern of NaOH recrystallized from aqueous solution is also reported for comparison. The patterns have been translated on the ordinate axis.
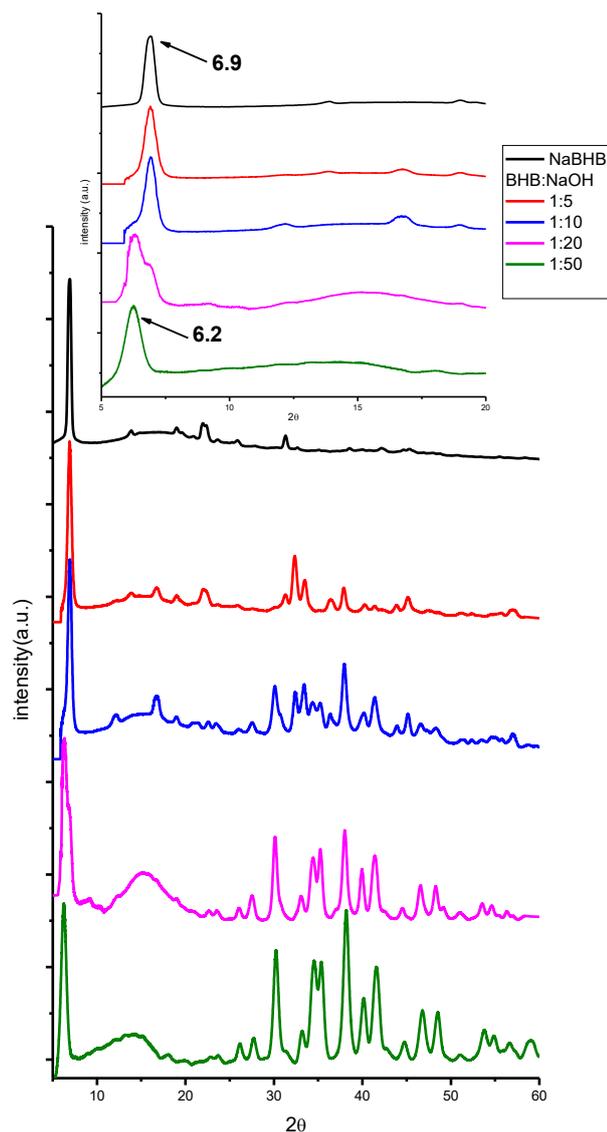
Figure A5. X-ray patterns of dry residues from the rum samples spiked with 1% v/v of BHB and BHB:NaOH molar ratios 1:5, 1:10, 1:20, 1:50. In the inset is evidenced the zone between 5 and 20 2θ values. The patterns have been translated on the ordinate axis and normalized with respect to the higher signal of NaBHB.

References

1   Elliott S.P. Gamma hydroxybutyric acid (GHB) concentrations in humans and factors affecting endogenous production. *Forensic Sci. Int.* **2003**, 133, 9-16. doi: 10.1016/s0379-0738(03)00043-4

2   Brailsford A.D.; Cowan D.A.; Kicman A.T. Pharmacokinetic properties of ⊚-hydroxybutyrate (GHB) in whole blood, serum, and urine. *J. Anal. Toxicol.* **2012**, 36, 88-95.

3   Wong C.G.; Chan K.F.; Gibson K.M.; Snead O.C. Gamma-hydroxybutyric acid: neurobiology and toxicology of a recreational drug. *Toxicol. Rev.* **2004**, 23(1), 3-20. doi: 10.2165/00139709-200423010-00002

4   Robinson D.; Keatin G.M. Sodium oxybate: A review of its use in the management of narcolepsy. *CNS Drugs* **2007**, 21(4), 337-54. doi:10.2165/00023210-200721040-00007

5   Boscolo-Berto R.; Viel G.; Montagnese S.; Raduazzo D.I.; Ferrara S.D.; Dauvilliers Y. Narcolepsy and effectiveness of gamma-hydroxybutyrate (GHB): a systematic review and meta-analysis of randomized controlled trials. *Sleep Med. Rev.* **2012**, 16, 431-443. doi: 10.1016/j.smrv.2011.09.001

6   Chick J.; Nutt D.J. Substitution therapy for alcoholism: Time for a reappraisal? *J. Psychopharmacol.* **2012**, 26(2), 205-12. doi:10.1177/0269881111408463

7   Keating G.M. Sodium oxybate: A review of its use in alcohol withdrawal syndrome and in the maintenance of abstinence in alcohol dependence. *Clin. Drug. Investig.* **2014**, 34(1), 63-80. doi:10.1007/s40261-013-0158-x

8   García M.G.; Pérez-Cárceles M.D-; Osuna E.; Legaz I. Drug-facilitated sexual assault and other crimes: A systematic review by countries. *J. Forensic. Leg. Med.* **2021**, 79, 102151. doi: 10.1016/j.jflm.2021.102151.

9   Kapitány-Fövény M.; Zacher G.; Posta J.; Demetrovics Z. GHB-involved crimes among intoxicated patients. *Forensic Sci. Int.* **2017,** 275, 23-29. doi: 10.1016/j.forsciint.2017.02.028.

10   Schwartz R.H.; Milteer R.; LeBeau M.A. Drug-facilitated sexual assault ('date rape'). *South Med J.* **2000**, 93(6), 558-61.

11   Shbair M.K.; Lhermitte M. Drug-facilitated crimes: definitions, prevalence, difficulties and recommendations. A review. *Ann. Pharm. Fr.* **2010**, 68(3), 136-47. doi: 10.1016/j.pharma.2010.03.005.

12   Németh Z.; Kun B.; Demetrovics Z. Review: The involvement of gamma-hydroxybutyrate in reported sexual assaults: a systematic review. *J. Psychopharmacol.* **2010**, 24(9), 1281-1287. doi:10.1177/0269881110363315.

13   Skov K.; Johansen S.S.; Linnet K.; Nielsen M.K.K. A review on the forensic toxicology of global drug-facilitated sexual assaults. *Eur. Rev. Med. Pharmacol Sci.* **2022**, 26(1), 183-197. doi: 10.26355/eurrev_202201_27767., 2022; 26: 183-197

14   Carter L.; Richards B.; Mintzer M.; Griffiths R.R. Relative Abuse Liability of GHB in Humans: A Comparison of Psychomotor, Subjective, and Cognitive Effects of Supratherapeutic Doses of Triazolam, Pentobarbital, and GHB. *Neuropsychopharmacol* **2006,** 31, 2537–2551. https://doi.org/10.1038/sj.npp.1301146

15   Hillebrand J.; Olszewski D.; Sedefov R. (**2008**). *GHB and its precursor GBL : an emerging trend case study* (European Monitoring Centre for Drugs and Drug Addiction, Ed.). Lisbon: European Monitoring Centre for Drugs and Drug Addiction (EMCDDA).

16   Corkery J.M.; Loi B.; Claridge H.; Goodair C.; Corazza O.; Elliott S.; Schifano F. Gamma hydroxybutyrate (GHB), gamma butyrolactone (GBL) and 1,4-butanediol (1,4-BD; BDO): A literature review with a focus on UK fatalities related to non-medical use. *Neurosci. Biobehav. Rev.* **2015**, 53, 52-78. doi: 10.1016/j.neubiorev.2015.03.012.

17   Casey T.; Lesar C.T.; Decatur J.; Lukasiewicz E.; Champeil E. Report on the analysis of common beverages spiked with gamma-hydroxybutyric acid (GHB) and gamma-butyrolactone (GBL) using NMR and the PURGE solvent-suppression technique. *Forensic Sci. Int.* **2011**, 212(1-3), e40-5. doi: 10.1016/j.forsciint.2011.06.017.

18   Beynon C.M.; McVeigh C.; McVeigh J.; Leavey C.; Bellis M.A. The involvement of drugs and alcohol in drug-facilitated sexual assault: a systematic review of the evidence. *Trauma Violence Abuse* **2008**, 9(3), 178-88. doi: 10.1177/1524838008320221. PMID: 18541699.

19   Busardò F.P.; Varì M.R.; di Trana A.; Malaca S.; Carlier J.; di Luca N.M. Drug-facilitated sexual assaults (DFSA): a serious underestimated issue. *Eur. Rev. Med. Pharmacol Sci.* **2019** 23(24), 10577-10587. doi: 10.26355/eurrev_201912_19753

20   Djezzar S.; Gaulier J.M.; Gorgiard C.; Chèze M.; Alvarez J.C.; Martin C.; Dumestre-Toulet, V.; Lavit M.; Mathieu O.; Eysseric H.; Berland E.; Roussel C.; Gaillard Y.; Hurtel-Lemaire A.S.; Deveaux M.; Pion C. Assessment of 19 years of a prospective national survey on drug-facilitated crimes in France, *Legal Medicine* **2023**, 65, 102297, https://doi.org/10.1016/j.legalmed.2023.102297.

21   Hagemann C.T.; Helland A.; Spigset O.; Espnes K.A.; Ormstad K.; Schei B. Ethanol and drug findings in women consulting a Sexual Assault Center – Associations with clinical characteristics and suspicions of drug-facilitated sexual assault, *J. Forensic Leg. Med.* **2013**, 20, 6, 777-784. https://doi.org/10.1016/j.jflm.2013.05.005.

22   Dufayet L.; Bargel S.; Bonnet A.; Boukerma A.K.; Chevallier C.;, Evrard M.; Guillotin S.; Loeuillet E.; Paradis C.; Pouget A.M.; Reynoard J.; Vaucel J.A. Gamma-hydroxybutyrate (GHB), 1,4-butanediol (1,4BD), and gamma-butyrolactone (GBL) intoxication: A state-of-the-art review. *Regul. Toxicol. Pharmacol.* **2023**, 142, 105435. doi: 10.1016/j.yrtph.2023.105435.

23   Ingels A.S.M.E.; Wille S.M.R.; Samyn N.; Lambert W.E.; Stove C.P.S. Screening and confirmation methods for GHB determination in biological fluids. Anal. Bioanal. Chem. **2014**, 406, 3553–3577. doi:10.1007/s00216-013-7586-6.

24   Dziadosz M.; Klintschar M.; Teske J. Signal-Separated Quantification of γ-Hydroxybutyrate with Liquid Chromatography-Tandem Mass Spectrometry in Human Urine and Serum as an Improvement of the Analyte Adduct Ion-Based Quantification. J. *Anal. Toxicol.* **2022**, 46(6), 676-682. doi: 10.1093/jat/bkab083.

25   Sørensen L.K.; Hasselstrøm J.B. A hydrophilic interaction liquid chromatography electrospray tandem mass spectrometry method for the simultaneous determination of γ-hydroxybutyrate and its precursors in forensic whole blood. *Forensic Sci. Int.* **2012**, 222(1-3):352-9. doi: 10.1016/j.forsciint.2012.07.017.

26   Kang S.; Oh S.M.; Chung K.H.; Lee S. A surrogate analyte-based LC-MS/MS method for the determination of γ-hydroxybutyrate (GHB) in human urine and variation of endogenous urinary concentrations of GHB. *J. Pharm. Biomed. Anal.* **2014**, 98, 193-200. doi: 10.1016/j.jpba.2014.05.028.

27   Yanshuxian L.; Yilei F.; Zhongping H.; Huijun L.; Lili W.; Zhenlu S.; Ichi W. Rapid determination of γ-hydroxybutyric acid in human hair by on-line pyrolytic methylation-gas chromatography/mass spectrometry. *J. Anal. Appl Pyrol.* **2020**, 151, 104920. doi:10.1016/j.jaap.2020.104920.

28   Steuer A.E.; Bavato F.; Schnider L.K.; Dornbierer D.A.; Bosch O.G.; Quednow B.B.; Seifritz E. Steuer C. Kraemer T.   Urinary concentrations of GHB and its novel amino acid and carnitine conjugates following controlled GHB administration to humans. *Sci. Rep.* **2023** 13, 8983. doi:/10.1038/s41598-023-36213-1.

29   Davis K.E.; Hickey L.D.; Goodpaster J.V. Detection of ɣ-hydroxybutyric acid (GHB) and ɣ-butyrolactone (GBL) in alcoholic beverages via total vaporization solid-phase microextraction (TV-SPME) and gas chromatography–mass spectrometry. *J. Forensic Sci.* **2021**, 66, 846–853. doi:10.1111/1556-4029.14660.

30   Elliott S, Burgess V. The presence of gamma-hydroxybutyric acid (GHB) and gamma-butyrolactone (GBL) in alcoholic and non-alcoholic beverages. Forensic Sci Int. **2005**, 151(2-3):289-92. doi: 10.1016/j.forsciint.2005.02.014.

31   Grootveld M.; Algeo D.; Silwood C.J.; Blackburn J.C.; Clark A.D. Determination of the illicit drug gamma-hydroxybutyrate (GHB) in human saliva and beverages by 1H NMR analysis. *Biofactors* **2006**, 27(1-4), 21-36. doi: 10.1002/biof.5520270111.

32   Chew S.L.; Meyers J.A. Identification and quantitation of gamma-hydroxybutyrate (NaGHB) by nuclear magnetic resonance spectroscopy. *J. Forensic Sci.* **2003**, 48(2), 292-8.

33   Del Signore A.G.; McGregor M.; Cho B.P. 1H NMR analysis of GHB and GBL: further findings on the interconversion and a preliminary report on the analysis of GHB in serum and urine. *J. Forensic Sci.* **2005** 50(1), 81-6.

34   Chappell J.S.; Meyn A.W.; Ngim K.K. The extraction and infrared identification of gamma-hydroxybutyric acid (GHB) from aqueous solutions. *J. Forensic Sci.* **2004**, 49(1), 52-9.

35   DeFrancesco J.V.; Witkowski M.R.; Ciolino L.A. GHB free acid: I. Solution formation studies and spectroscopic characterization by 1HNMR and FT-IR. *J. Forensic Sci.* **2006**, 51(2), 321-9. doi: 10.1111/j.1556-4029.2006.00073.x.

36   Ciolino L.A; Mesmer M.Z.; Satzger R.D.; Machal A.C.; McCauley H.A.; Mohrhaus A.S. The chemical interconversion of GHB and GBL: forensic issues and implications. *J. Forensic Sci.* **2001**, 46(6), 1315-23.

37   Witkowski M.R; Ciolino L.A.; Defrancesco J.V. GHB free acid: II. Isolation and spectroscopic characterization for forensic analysis. *J. Forensic Sci.* **2006**, 51(2), 330-9. doi: 10.1111/j.1556-4029.2006.00074.x.

38   Kranenburg R.F.; Weesepoel Y.; Alewijn M.; Sap S.; Arisz P.W.F.; van Esch A.; Keizers P.H.J.; van Asten A.C. The importance of wavelength selection in on-scene identification of drugs of abuse with portable near-infrared spectroscopy, *Forensic Chemistry*, **2022**, 30, 100437. doi: 10.1016/j.forc.2022.100437.

39   West M.J.; Went M.J. Detection of drugs of abuse by Raman spectroscopy. *Drug Test. Anal.* **2011**, 3(9), 532-8. doi: 10.1002/dta.217.

40   Brewster V.L.; Edwards H.G.; Hargreaves M.D.; Munshi T. Identification of the date-rape drug GHB and its precursor GBL by Raman spectroscopy. *Drug Test. Anal.* **2009**, 1(1), 25-31. doi: 10.1002/dta.11.

41   Inscore F.; Shende C.; Sengupta A.; Huang H.; Farquharson S. Detection of drugs of abuse in saliva by surface-enhanced Raman spectroscopy (SERS). *Appl. Spectrosc.* **2011**, 65(9), 1004-8. doi: 10.1366/11-06310.

42   Alston W.C. 2nd; Ng K. Rapid colorimetric screening test for gamma-hydroxybutyric acid (liquid X) in human urine. *Forensic Sci Int.* **2002**, 126(2), 114-117. doi: 10.1016/s0379-0738(02)00050-6.

43   Soni S.; Jain U.; Chauhan N. A systematic review on sensing techniques for drug- facilitated sexual assaults (DFSA) monitoring. *Chinese J. Anal. Chem.* **2021**, 49, 83-92. doi: 10.1016/j.cjac.2021.09.001.

44   Xu W.; Zhai D.; Chang Y.-T., Chapter 49 - Detection of GHB by Optical Methods, in Neuropathology of Drug Addictions and Substance Misuse, Editor(s): Victor R. Preedy; Publisher: Academic Press, 2016, Volume 2, pp. 529-535.

45   Rodríguez-Nuevalos S.; Costero A.M.; Parra M.; Gil S.; Arroyo P.; Saez J.A.; Gaviña P.; Ceroni P.; Fermi A. Colorimetric and fluorescent hydrazone-BODIPY probes for the detection of γ-hydroxybutyric acid (GHB) and cathinones. *Dyes and Pigments* **2022**, 207, 110757. doi: 10.1016/j.dyepig.2022.110757.

46   Busardò F. P.; Jones A.W. GHB pharmacology and toxicology: acute intoxication, concentrations in blood and urine in forensic cases and treatment of the withdrawal syndrome. *Curr. Neuropharmacol.* **2015**, 13(1), 47-70. doi: 10.2174/1570159X13666141210215423.

47   Scharf M.B.; Lai A.A.; Branigan B.; Stover R.; Berkowitz D.B. Pharmacokinetics of gammahydroxybutyrate (GHB) in narcoleptic patients. *Sleep*, **1998**, 21, 507-514.

48   Abanades S.; Farré M.; Segura M.; Pichini S.;   Barral D.; Pacifici R.; Pellegrini M.; Fonseca F.;   Langohr K.; De La Torre R. Gamma-hydroxybutyrate (GHB) in humans: pharmacodynamics and pharmacokinetics. *Ann. N. Y. Acad. Sci.* **2006**, 1074, 559-576. doi: 10.1196/annals.1369.065.

49   Guidelines for the Forensic analysis of drugs facilitating sexual assault and other criminal acts- United Nations Office on Drugs and Crime; United Nations: New York, 2011, pp. 2011-300.

50   Bennett M.J.; Steiner R.R. Detection of gamma-hydroxybutyric acid in various drink matrices via AccuTOF-DART. *J. Forensic Sci.* **2009**, *54(2),370-5. doi: 10.1111/j.1556-4029.2008.00955.x*

51 Meng L.; Chen S.; Zhu B.; Zhang J.; Mei Y.; Cao J.; Zheng K. Application of dispersive liquid-liquid microextraction and GC-MS/MS for the determination of GHB in beverages and hair. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.* **2020**, 1144, 122058. doi: 10.1016/j.jchromb.2020.122058.

52 Junghyun R.; Youngmi K. Overcoming interferences in the colorimetric and fluorimetric detection of γ-hydroxybutyrate in spiked beverages. *Sensors & Actuators B: Chemical* **2022**, 364, 131861.

53 Jin S.; Ning X.; Cao J.; Wang Y. Simultaneous Quantification of γ-Hydroxybutyrate, γ-Butyrolactone, and 1,4-Butanediol in Four Kinds of Beverages. *Int. J. Anal. Chem.* **2020**, 8837743, pp.7. doi: 10.1155/2020/8837743.

54 Li Y.; Wang L.; Qian M.; Qi S.; Zhou L.; Pu Q. Concise analysis of γ-hydroxybutyric acid in beverages and urine by capillary electrophoresis with capacitively coupled contactless conductivity detection using 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid as background electrolyte. *J. Chromatogr. A.* **2022**, 1675, 463191. doi: 10.1016/j.chroma.2022.463191.

55 Meyers J.; Almirall J.R. Analysis of gamma-hydroxybutyric acid (GHB) in spiked water and beverage samples using solid phase microextraction (SPME) on fiber derivatization/gas chromatography-mass spectrometry (GC/MS). *J. Forensic Sci.* **2005**, 50(1), 31-6. doi:10.1520/JFS2003280.

56 Munshi T.; Brewster V.L.; Edwards H.G.; Hargreaves M.; Jilani S.K.; Scowen I.J. Monitoring of the interconversion of gamma-butyrolactone (GBL) to gamma hydroxybutyric acid (GHB) by Raman spectroscopy. *Drug Test. Anal.* **2013**, 5(8), 678-82. doi: 10.1002/dta.1438.

57 Kugler, W. X-ray diffraction analysis in the forensic science: The last resort in many criminal cases. *Adv. X-ray* Anal. **2003**, 46, 1-16.

58 Rendle D.F. Forensic applications of X-ray powder diffraction. *International Tables for Crystallography* **2019**. vol H. ch. 7.2, pp. 737-751.   https://doi.org/10.1107/97809553602060000976

59 Cappelletti P.; Graziano S.F.; Bish D.L. (2023). X-ray Diffractometry in Forensic Science. In Mineralogical Analysis Applied to Forensics. Soil Forensics.; Mercurio M., Langella, A., Di Maggio, R.M., Cappelletti, P. Eds.;   Springer, Cham. https://doi.org/10.1007/978-3-031-08834-6_2

60 Kotrlý, M. Using X-ray diffraction in forensic science *Zeitschrift für Kristallographie - Crystalline Materials*, **2007**, 222(3-4), 193-198. https://doi.org/10.1524/zkri.2007.222.3-4.193

61 Abraham J.T.; Shukla S. K.; Singh A. K. Application of X-Ray Diffraction Techniques in Forensic Science. *Forensic Sci. Commun.* **2007**, 9, 1-6.

62 Jurásek B.; Bartůněk V.; Huber Š.; Kuchař M. X-Ray powder diffraction – A non-destructive and versatile approach for the identification of new psychoactive substances, *Talanta* **2019**, 195, 414-418, https://doi.org/10.1016/j.talanta.2018.11.063.

63 Jurásek B.; Bartůněk V.; Huber Š.; Fagan P.; Setnička V.; Králík F.; Dehaen W.; Svozil D.; Kuchař M. Can X-Ray Powder Diffraction Be a Suitable Forensic Method for Illicit Drug Identification?. *Front. Chem.* **2020**, 8, 499. https://doi.org/10.3389/fchem.2020.00499

64 Bulska E.; Bachliński R.; Cyrański M.K.; Michalska-Kacymirow M.; Kośnik W.; Małecki P.; Grela K.; Dobrowolski M.A. Comprehensive Protocol for the Identification and Characterization of New Psychoactive Substances in the Service of Law Enforcement Agencies. *Front. Chem.* **2020**, 8, 693. https://doi.org/10.3389/fchem.2020.00693

65 Decreto del Presidente della Repubblica 9 ottobre, n. 309. Testo unico delle leggi in materia di disciplina degli stupefacenti e sostanze psicotrope, prevenzione, cura e riabilitazione dei relativi stati di tossicodipendenza.Gazzetta Ufficiale – Serie Generale n. 255 (Suppl. Ordinario n. 67), 31 ottobre 1990.

66 Di Luca N.M.; Busardò F.P.; Pirani F.; Varì M.R. Evolution of Italian laws banning trafficking, use and abuse of psychotropic drugs. *Ann. Ist. Supe.r Sanità* **2020**, 56(1), 76-89. doi: 10.4415/ANN_20_01_12. PMID: 32242539.

66 Gorecho E.N. Qualitative analysis of sodium gamma-hydroxybutyric acid (GHB) residue from plastic and glass containers using FT-IR ATR, thesis in Master of Science in Forensic Science in the Office of graduate studies of the University of California, UMI Number: 1502266, 2011;

67 Ferris T.J.; Went M.J. Synthesis, characterisation and detection of γ-hydroxybutyrate salts. Forensic Sci. Int. **2012**; 216(1-3):158-62. doi: 10.1016/j.forsciint.2011.09.014.

68 Elliott S.P.; Fais P. Further evidence for GHB naturally occurring in common non-alcoholic beverages. *Forensic Sci. Int.* **2017**, 277, e36-e38. doi: 10.1016/j.forsciint.2017.05.012.

69 Tucci M.; Stocchero G.; Pertile R.; Favretto D. Detection of GHB at low levels in non-spiked beverages using solid phase extraction and gas chromatography–mass spectrometry, *Toxicologie Analytique et Clinique* **2017**, 29, 225-233. https://doi.org/10.1016/j.toxac.2016.12.009.

70 Payan C.; Gancel A.-L.; Jourdes M.; Christmann M.;   Teissedre P.-L. Wine acidification methods: a review. *OENO One* **2023**, 57(3), 113–126. https://doi.org/10.20870/oeno-one.2023.57.3.7476

71 Chemical Composition of Alcoholic Beverages, Additives and Contaminants. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Alcohol Drinking. Lyon (FR): International Agency for Research on Cancer; 1988. (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 44.). https://www.ncbi.nlm.nih.gov/books/NBK531662/

23