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TECHNICAL NOTE

CRIMINALISTICS; TOXICOLOGY

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Rapid Screening for the Detection and Differentiation of Gamma-Hydroxybutyrate using Ion Chromatography*,†

ABSTRACT: The analysis of *gamma*-hydroxybutyric acid (GHB) is problematic because it is hygroscopic, it lacks a good UV chromophore, and it undergoes heat-induced cyclization. This paper presents a new method utilizing ion-exchange chromatography (IC) with conductivity detection. The simple sample preparation, rapid analysis time, and inorganic anion detection capabilities are all advantages over the current methods. The detection of inorganic salts (formed during GHB synthesis) gives insight into the synthetic route utilized and can aid in drug seizure comparison. The developed method has a detection limit for GHB anions of 0.57 mg/L and chloride of 0.22 mg/L. A comparison of this technique with a current gas chromatography–mass spectrometry technique is presented, and a *t*-test found that the two methods' results are not statistically different at the 99.9% confidence level demonstrating the merits of this fast, simple, and informative IC method as a routine screening tool.

KEYWORDS: forensic science, gamma-hydroxybutyrate, gamma-butyrolactone, ion-exchange chromatography, illicit drugs, gas chromatography–mass spectrometry, GHB, GBL, GC-MS

Gamma-hydroxybutyric acid (GHB) occurs naturally in mammalian species and is thought to have neurotransmitter or neuromodulator properties (1). It has been used medically in the treatment of narcolepsy (2), to induce anesthesia (3), and in the management of alcoholism (4) and opiate withdrawal (5). Over recent years, GHB has been used illicitly as a recreational drug and is known for its connection with sexual assaults facilitated by spiked drinks (1,6,7). The illicit use of GHB is particularly dangerous owing to its steep dose–response curve, which increases the risk of accidental overdose (8).

The synthesis of GHB is readily achieved by mixing *gamma*-butyrolactone (GBL) with a strong alkaline base such as sodium hydroxide, followed by neutralization with an acid (most commonly hydrochloric acid). The resulting GHB solution contains a salt by-product, which varies depending on the acid used. In the case of hydrochloric acid, a sodium chloride by-product results (1). Methods for the synthesis are readily available on the internet.

GHB is commonly analyzed using gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS) (9–16). However, these methods are problematic as the hot injection port of the GC causes heat-induced cyclization of GHB into its lactone form (GBL), thus affecting the limits of detection (LOD), limits of quantitation (LOQ), and reproducibility (17). GC methods can therefore require extensive sample preparation to inhibit the cyclization and increase detection sensitivity. Trimethyl silyl derivatization using bis(trimethyl-silyl) trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) is the most commonly utilized sample preparation technique and has been used for the determination of GHB in hair, blood, saliva, urine, and beverages (12–16). Alternative derivatization reagents such as trifluoroacetic anhydride with 2,2,3,3,4,4,4-heptafluoro-1-butanol (2:1 v/v) (11), *N*-(tert-butyldimethylsilyl)-*N*-methyltrifluoroacetamide (MTBSTFA) (9), hexyl–chloroformate with pyridine (1:4 v/v) (10), and *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) (18) have also been utilized for the derivatization of GHB.

Another approach for the GC determination of GHB involves sample acidification to convert all GHB into the lactone form (GBL) (Fig. 1) and the subsequent analysis of GBL (19–21).

Simpler approaches requiring limited sample preparation (dilution) have included high-performance liquid chromatography (HPLC) and nuclear magnetic resonance spectroscopy (NMR). Mesmer et al. (22) successfully utilized HPLC/ultraviolet–visible spectrophotometry and HPLC/MS for the separation and quantitation of GHB and GBL in illegal preparations. Chew et al. (23) used proton and carbon NMR to analyze samples of GHB, GBL, and mixtures of the two.

While all of these methods can be used to determine GHB in a sample, none of them are capable of detecting the sodium chloride by-product. Sodium chloride can be found at high concentrations in GHB preparations and because of it being an inorganic salt, its simultaneous detection with GHB by analytical instruments is limited. By simultaneously analyzing the salt by-product concentration

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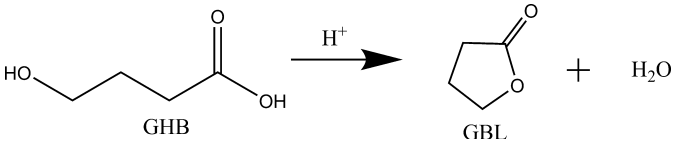

FIG. 1—*The acid catalyzed conversion of GHB into GBL.*

and the GHB anion concentration, the comparison of GHB seizures can be undertaken for drug profiling purposes. Identifying the salt by-product formed during synthesis can also inform on the chemicals utilized in the clandestine manufacture of the illicit drug, providing important intelligence to law enforcement agencies. Owing to the ionic nature of both NaGHB and NaCl, separation and detection using an ion-exchange chromatography (IC) system are possible. Here, we report an IC method utilizing conductivity detection that is capable of detecting the anion of GHB as well as the chloride anion by-product with minimal sample preparation.

IC is used extensively in the food and beverages industry (24), for water quality testing (25,26), atmospheric monitoring (27,28), and in the pharmaceutical industry (29). Examples of forensic applications include explosives analysis (30), soil analysis (31), adultered urine determination (32), drug authentication (33), and drug profiling (34). IC is a well-established technique and has been the preferred method for the detection of inorganic anions and small organic anions for the past 20 years (35), making it an ideal technique for the application to GHB detection.

To our knowledge, the application of IC for the detection of GHB has not been reported and this is the first application for the simultaneous analysis of the GHB anion and chloride. The developed IC technique is able to determine GHB purity quickly and easily with no sample pretreatment other than dilutions and filtering.

Materials and Methods

Chemicals

Gamma-butyrolactone (GBL) (98.5%) was purchased from Koch Light Laboratories (Suffolk, England). Sodium chloride (99.9%), L-ascorbic acid (99%), and hydrochloric acid (36%) were purchased from Asia Pacific Specialty Chemicals Limited (Seven Hills, NSW, Australia). Sodium bromide (99%), sodium hydroxide (98%), sodium carbonate (99.8%), sodium bicarbonate (99%), and ethanol (analytical grade) were purchased from Chem-Supply (Gillman, SA, Australia). Sodium *gamma*-hydroxybutyrate (NaGHB) (99%) was purchased from National Measurement Institute (NMI) (Pymble, NSW, Australia). Deuterated NaGHB-d_6 (1 mg/mL in methanol) was purchased from Cerilliant (Round Rock, TX), BSTFA with 1% TMCS, potassium benzoate, glycolic acid (99%), and ammonium formate (99.995%) were purchased online from Sigma Aldrich. Sodium fluoride (99%), sodium nitrite (97%), ammonium nitrate (99%), sodium dihydrogen orthophosphate (99%), ammonium acetate (96%), and salicylic acid (99.5%) were purchased from Ajax chemicals (Sydney, NSW, Australia). Sodium borate was purchased from A.C. Hatrick Chemicals (Botany, NSW, Australia). Ammonium bromate (99%), succinic acid, ammonium tartrate (99%), ammonium oxalate (99%), and tri-sodium citrate (99%) were obtained from the British Drug House Laboratories (Poole, U.K.). Sodium sulfate (99%) was purchased from Merck (Kilsyth, Vic., Australia), and water was ultra purified to 18 megohm cm using a Barnstead™ E-Pure™ (Thermo Fisher Scientific, Waltham, MA) water system.

Synthesis of GHB

NaGHB was synthesized in the laboratory by boiling GBL (2 mL) with sodium hydroxide (0.92 g) and aqueous ethanol (40% [v/v], 3 mL) under reflux for 1 h (36). Hydrochloric acid was added dropwise until the solution reached pH 7. The neutralized solution was then left to stand at room temperature (25°C) overnight, resulting in NaGHB precipitation. The solid NaGHB salt was then filtered under vacuum and was stored in a desiccator until analysis. Eight different batches of NaGHB were synthesized and are referred to as GHB 1–GHB 8.

Sample Preparation

Ion Chromatography Samples—Chloride calibration standards were prepared by dissolving sodium chloride in mobile phase (1 mM $NaHCO_3$/8 mM Na_2CO_3) and made up to the required concentrations with an internal standard concentration of 20 mg/L bromide ion. Fifty microliters of each chloride standard was injected onto the IC column in triplicate. Chloride peak areas relative to the bromide internal standard peak areas were averaged over the replicates and plotted against chloride concentration.

GHB anion calibration standards for IC analysis were prepared from serial dilutions of a GBL standard with mobile phase. An internal standard concentration of 20 mg/L bromide ion was added. To ensure the complete conversion from the lactone into the anion, the standards were left in a 70°C oven for 30 min prior to injecting 50 μL onto the IC column in triplicate. GHB anion peak areas relative to the bromide internal standard peak areas were averaged over the replicates and plotted against GHB anion concentration.

Solutions of the laboratory synthesized NaGHB salts were made by dissolving *c.* 5 mg of dried drug in 50 mL of mobile phase with 20 mg/L bromide internal standard such that the final NaGHB concentration was *c.* 100 mg/L. Fifty-microliter aliquots of these solutions were then injected onto the column in triplicate.

All salts involved in the interfering anion study were dissolved in mobile phase (*c.* 20 mg/L), before 50 μL was injected onto the IC column.

Gas Chromatography–Mass Spectrometry Samples—To prepare NaGHB for GC-MS analysis, dry NaGHB was accurately weighed and dissolved in methanol. Thirty microliters of this solution, together with 3 μL of deuterated NaGHB internal standard solution, was evaporated to dryness under a stream of nitrogen. The dried sample was derivatized with 30 μL of BSTFA with 1% TMCS and incubated in a 70°C oven for 30 min. One microliter of this solution was injected onto the GC-MS column. Both standards and samples were prepared in this way; however, NaGHB standard solutions were prepared with NMI NaGHB to concentrations between 1.5 and 60 mg/L, and synthesized NaGHB samples were prepared to concentrations of *c.* 30 mg/L.

Instrumentation

Ion Chromatography—Ion chromatography was performed on a Dionex ICS-1500 ion chromatography system (Dionex Corporation, Sunnyvale, CA) composed of a pump, AS40 auto sampler, column heater, and conductivity detector. The column was a Dionex Ionpac® AS14A anion-exchange column (4 × 250 mm and 7 μm bead diameter). The mobile phase, 1 mM sodium bicarbonate/8 mM sodium carbonate in e-pure water (pH 10), filtered with a 0.45-μm nylon membrane was used with a flow rate of 0.7 mL/min and with isocratic elution at 40°C. The suppressor

system was a Dionex ASRS 300 (4 mm) anion self-regenerating suppressor operated at 35°C.

Gas Chromatography–Mass Spectrometry—Validation of the IC technique was performed on a Varian Saturn 2200 GC-MS-MS (Varian Inc., Palo Alto, CA). A 1-μL aliquot of the derivatized sample was injected into the column of a Varian gas chromatograph (CP-3800 GC). The flow of the carrier gas (helium) through the Zebron (Zebron Corporation, Newport Beach, CA) column (ZB-5MS capillary column, 5%-phenyl-arylene-95%-dimethylpolysiloxane, 30 m × 0.25 mm I.D × 0.25 μm film thickness) was 2 mL/min. The injector temperature was 270°C with a split ratio of 50:1 being employed. The column oven temperature was programmed at 70°C for the first minute, then +10°C/min to 100°C and held at 100°C for the final 6 min. The detector was a Varian 2000 Series Ion Trap MS operated in electron ionization mode. The trap, manifold, and transfer line temperatures were 200, 120, and 250°C, respectively, and the electron multiplier was operated at 1700 V.

Data were recorded in full scan (40–400 *m/z*), and ions monitored were the following: *m/z* 233, 204, and 147 and *m/z* 239 for GHB and GHB-d_6, respectively (the underlined ions used for quantitation).

Results and Discussion

Method Development

Interfering Anions—The IC method was optimized to give the best separation between the GHB anion and various common inorganic anions. Figure 2 shows the seven anions analyzed and their baseline separation from the GHB anion. Numerous organic anions were also included in the study as shown in Table 1. Most anions were baseline separated from the GHB anion with the exceptions of acetate and formate that co-eluted with the drug. The presence of these anions in a NaGHB seizure is possible if acetic acid or formic acid is used instead of hydrochloric acid for the neutralization step during synthesis. Acetate and formate can also be present in beverages into which GHB is commonly dissolved (16). These interfering anions, therefore, demonstrate the methods susceptibility to false positives.

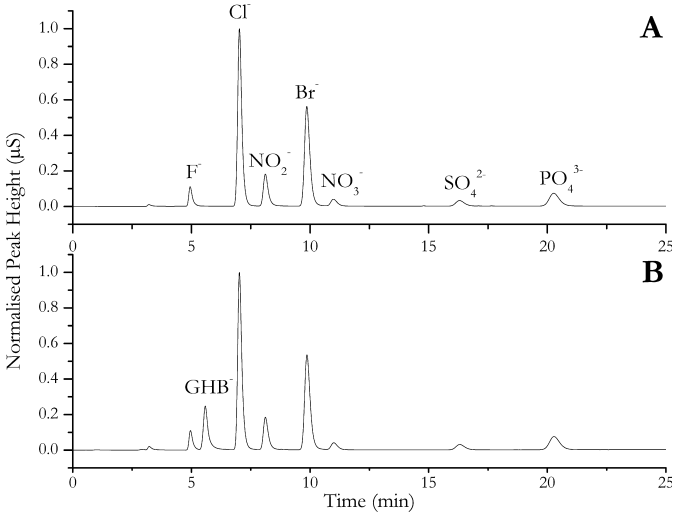

FIG. 2—*(A) The chromatogram of a seven anion mixture of common inorganic anions. (B) The same seven anion mixture spiked with a solution of NaGHB.*

TABLE 1—*The retention times of the organic anions included in the study.*

Anions	Retention Time (min)
Ascorbate	4.77
Glycolate	4.85
GHB	5.12
Acetate	5.17
Formate	5.28
Bromate	6.53
Salicylate	9.55
Succinate	12.53
Tartrate	14.60
Oxalate	18.28
Benzoate	50.49
Citrate	107.99
Borate	>120

Calibration—NaGHB is extremely hygroscopic, making the drug difficult to weigh accurately (23). For this reason, GBL was used to prepare the GHB anion calibration standards owing to its complete conversion into the GHB anion at mobile phase pH (37). The resulting GHB anion calibration line ($y = 0.0062x + 0.0587$) was linear ($r^2 = 0.9986$) over the 3–135 mg/L range. The percent relative standard deviations (CV) ranged from 0.5 to 6.2% for the replicates ($n = 3$).

The limit of detection for each anion was determined using equation 1:

$$\text{LOD or LOQ} = \frac{KS_b}{m} \quad (1)$$

where K is the confidence factor, S_b is the standard deviation of the blank, and m is the calibration sensitivity (the slope of the calibration graph). Confidence factors of 3 and 10 were used to calculate the limit of detection (LOD) and the limit of quantitation (LOQ), respectively. The method for detecting the GHB anion using IC had a LOD of 0.57 mg/L and a LOQ of 1.9 mg/L.

The chloride calibration line ($y = 0.2186x - 0.6701$) was linear over the 1–210 mg/L calibration range ($r^2 = 0.9990$), with a LOD of 0.22 mg/L and a LOQ of 0.73 mg/L. The percent relative standard deviation between replicate analyses ranged from 0.25 to 1.86% for the replicates ($n = 3$), which indicates the high reproducibility of this developed method.

Differentiation of GHB Samples

Triplicate analysis of the laboratory synthesized NaGHB samples, with reference to the calibration lines, allowed the GHB anion and chloride concentrations of each sample to be determined.

Figure 3 shows that differentiation of the eight laboratory synthesized batches based on % GHB anion (w/w) was achieved after IC analysis with concentrations ranging from <5% (GHB-8) to >75% (GHB-1). However, the technique's ability to detect chloride and other anions simultaneously adds significant discriminatory power to the method. Figure 3 shows how GHB-2, GHB-7, and GHB-8 contain small amounts of the GHB anion (<10%) and large amounts of chloride (>30%). This amount of information about the purity of a NaGHB sample was achieved in 11 min with simple sample preparation (dilution and filtration). Reaction times and periods of crystallization may have varied between batches resulting in the range of compositions seen in Fig. 3. It is believed that this level of variation would occur in clandestinely synthesized NaGHB preparations making the

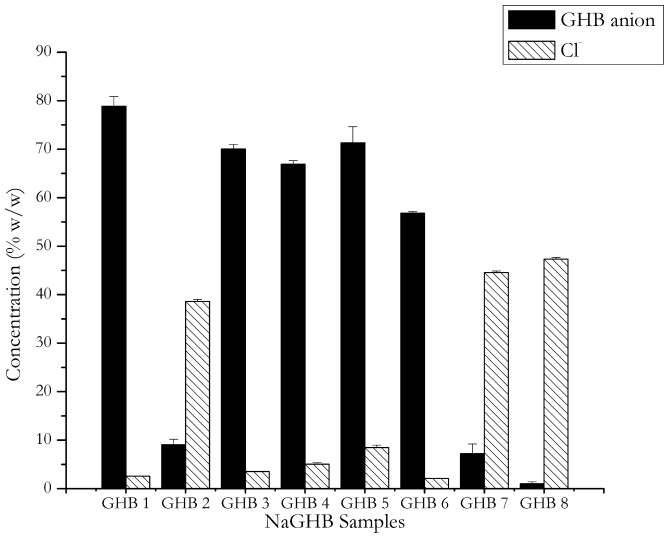

FIG. 3—*The IC results showing the compositions of each laboratory-synthesized NaGHB preparation. Error bars represent one standard deviation from the mean.*

simultaneous analysis of the GHB anion and chloride useful to forensic investigations.

Method Validation

The developed IC method was validated using a well-established GC-MS method analysing the GHB-TMS derivatives (12). All NaGHB samples were analyzed using both methods with results plotted against each other as shown in Fig. 4. The GC-MS results showed good agreement with the IC results for the eight NaGHB batches. The line, $y = x$, represents perfect correlation between the two methods, and most of the samples lie on this line or have error bars that cross the line. However, GHB-4 and GHB-6 deviated slightly from the line. The larger IC % GHB anion result of these two samples could be explained by the presence of coeluting anions in the sample or more likely, the presence of GBL in the sample converting into GHB anion in the mobile phase prior to analysis. Despite the slight deviation from the line for these two NaGHB samples, a *t*-test found that the two methods results are not statistically different at the 99.9% confidence level.

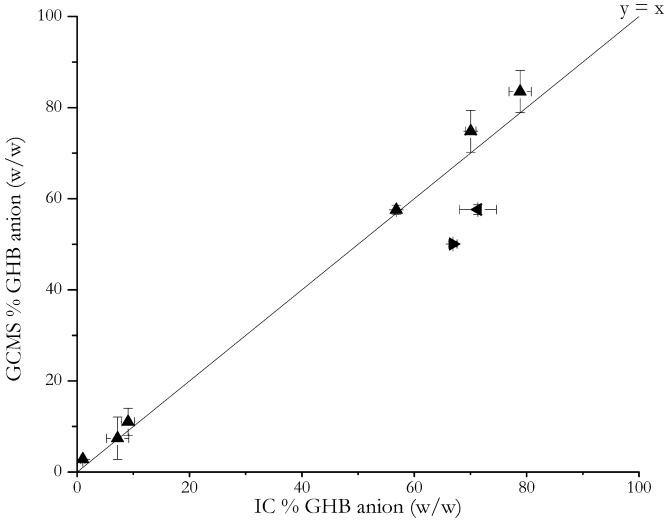

FIG. 4—*The correlation between the IC results and the GC-MS results with line y = x displayed, representing perfect correlation. GHB 4 (▶) and GHB 6 (◀) deviate slightly from the line. Error bars represent one standard deviation from the mean.*

Interestingly, when consumed, GBL is converted into GHB through metabolic processes, and therefore, GBL has the same biological effects over the body as GHB does (8). The developed IC method (in which all GBL present in a sample is converted into the GHB anion) is therefore reporting on biologically available GHB anion in a sample, rather than immediate GHB anion concentration, and is therefore a more accurate determination of sample potency than alternative methods. However, the law surrounding possession of GHB is different to that surrounding GBL in many jurisdictions meaning the accurate analysis of a sample's GHB content can be necessary.

Conclusion

The developed IC method has an advantage over established GC-MS procedures because of its simple sample preparation, rapid analysis time, and inorganic anion detection capabilities. Despite the simplicity of the method, reported GHB anion concentrations are in agreement with those determined using the GC-MS derivatization technique. However, the presence of GBL in the NaGHB sample will cause an overestimation of GHB anion concentration when analyzed using the IC method. Although this enables the biological potency of the sample to be determined, the IC method could be modified to enable the exclusive detection of both GHB anion and GBL by the addition of a liquid/liquid extraction step to the sample preparation before IC analysis. Further modifications to the method by the incorporation of an alternative stationary phase capable of resolving the acetate, formate, and GHB anion peaks could improve the method by eliminating the risk of false positives.

The reported IC method is therefore well suited as a screening tool. The limits of detection were 0.57 mg/L and 0.22 mg/L for the GHB anion and chloride respectively, making the method sensitive enough for the analysis of clandestine preparations. This method also enables the simultaneous detection of chloride ion that has the potential to be one of the most concentrated by-products in NaGHB preparations and can be a useful marker for batch-to-batch variation. As such, the developed method has the potential not only to be useful for the determination of GHB anion concentration in a sample but also to help identify samples from the same batch.

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References

1. Ferrara SD, Frison G, Tedeschi L, LeBeau M. Gamma-hydroxybutyrate (GHB) and related products. In: LeBeau MA, Mozayani A, editors. Drug-facilitated sexual assault: a forensic handbook. San Diego, CA: Academic Press, 2001;107–26.
2. Mamelak M, Scharf MB, Woods M. Treatment of narcolepsy with gamma-hydroxybutyrate. A review of clinical and sleep laboratory findings. Sleep 1986;9(1 Pt 2):285–9.
3. Kleinschmidt S, Schellhase C, Mertzlufft F. Continuous sedation during spinal anaesthesia: gamma-hydroxybutyrate vs. propofol. Eur J Anaesthesiol 1999;16(1):23–30.

4. Polodrugo F, Addolorato G. The role of gamma-hydroxybutyrate in the treatment of alcoholism: from animals to clinical studies. Alcohol Alcohol 1999;34(1):15–24.
5. Gallimberti L, Cibin M, Pagnin P, Sabbion R, Pani PP, Pirastu R, et al. Gamma hydroxybutyric acid for the treatment of opiate withdrawal syndrome. Neuropsychopharmacology 1993;9(1):77–81.
6. Pope E, Shouldice M. Drugs and sexual assault: a review. Trauma Violence Abuse 2001;2(1):51–5.
7. Dietze PM, Cvetkovski S, Barratt MJ, Clemens S. Patterns and incidence of γ-hydroxybutyrate (GHB)-related ambulance attendances in Melbourne, Victoria. Med J Aust 2008;188(12):709–11.
8. Couper FJ, Marinetti LJ. Gamma-hydroxybutyrate (GHB)—effects on human performance and behaviour. Forensic Sci Rev 2002;14(1/2):101–21.
9. Richard D, Ling B, Authier N, Faict TW, Eschalier A, Coudore F. GC/MS profiling of gamma-hydroxybutyrate and precursors in various animal tissues using automatic solid-phase extraction. Preliminary investigations of its potential interest in postmortem interval determination. Anal Chem 2005;77(5):1354–60.
10. Brown SD, Rhodes DJ, Pritchard BJ. A validated SPME-GC-MS method for the simultaneous quantification of club drugs in human urine. Forensic Sci Int 2007;171(2007):142–50.
11. Sabucedo AJ, Furton KG. Extractionless GC/MS analysis of gamma-hydroxybutyrate and gamma-butyrolactone with trifluoroacetic anhydride and heptafluoro-1-butanol from aqueous samples. J Sep Sci 2004; 27(9):703–9.
12. Villain M, Cirimele V, Ludes B, Kintz P. Ultra-rapid procedure to test for gamma-hydroxybutyric acid in blood and urine by gas chromatography-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2003;792(1):83–7.
13. Kintz P, Cirimele V, Jamey C, Ludes B. Testing for GHB in hair by GC/MS/MS after a single exposure. Application to document sexual assault. J Forensic Sci 2003;48(1):1–6.
14. Kintz P, Villain M, Cirimele V, Ludes B. GHB in postmortem toxicology discrimination between endogenous production from exposure using multiple specimens. Forensic Sci Int 2004;143(2004):177–81.
15. Kintz P, Goulle J-P, Cirimele V, Ludes B. Window of detection of gamma-hydroxybutyrate in blood and saliva. Clin Chem 2001;47(11): 2033–4.
16. Meyers JE, Almirall JR. Analysis of gamma-hydroxybutyric scid (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):1–6.
17. Hennessy SA, Moane SM, McDermott SD. The reactivity of gamma-hydroxybutyric acid (GHB) and gamma-butyrolactone (GBL) in alcoholic solutions. J Forensic Sci 2004;49(6):1–9.
18. Kankaanpaa A, Liukkonen R, Ariniemi K. Determination of gamma-hydroxybutyrate (GHB) and its precursors in blood and urine samples: a salting-out approach. Forensic Sci Int 2007;170(2007):133–8.
19. LeBeau MA, Montgomery MA, Morris-Kukoski C, Schaff JE, Deakin A, Levine B. A comprehensive study on the variations in urinary concentrations of endogenous gamma-hydroxybutyrate (GHB). J Anal Toxicol 2006;30(2):98–105.
20. Frison G, Tedeschi L, Maietti S, Ferrara SD. Determination of gamma-hydroxybutyric acid (GHB) in plasma and urine by headspace solid-phase microextraction and gas chromatography/positive ion chemical ionization mass spectrometry. Rapid Commun Mass Spectrom 2000; 14(24):2401–7.
21. Jones AW, Holmgren A, Kugelberg FC. Gamma-hydroxybutyrate concentrations in the blood of impaired drivers, users of illicit drugs, and medical examiner cases. J Anal Toxicol 2007;31(November/December): 566–72.
22. Mesmer MZ, Satzger RD. Determination of gamma-hydroxybutyrate (GHB) and gamma-butyrolactone (GBL) by HPLC/UV-VIS spectrophotometry and HPLC/thermospray mass spectrometry. J Forensic Sci 1998;43(3):489–92.
23. Chew SL, Meyers JA. Identification and quantitation of gamma-hydroxybutyrate (NAGHB) by nuclear magnetic resonance spectroscopy. J Forensic Sci 2003;48(2):1–7.
24. Zhu Y, Guo Y, Ye M, James FS. Separation and simultaneous determination of four artificial sweeteners in food and beverages by ion chromatography. J Chromatogr A 2005;1085(1):143–6.
25. Gros N, Camoes MF, Oliveira C, Silva MCR. Ionic composition of seawaters and derived saline solutions determined by ion chromatography and its relation to other water quality parameters. J Chromatogr A 2008; 1210(1):92–8.
26. Bruzzoniti MC, De Carlo RM, Fungi M. Simultaneous determination of alkali, alkaline earths and ammonium in natural waters by ion chromatography. J Sep Sci 2008;31(18):3182–9.
27. Vincent G, Kopferschmitt-Kubler MC, Mirabel P, Pauli G, Millet M. Sampling and analysis of quaternary ammonium compounds (QACs) traces in indoor atmosphere. Environ Monit Assess 2007;133(1–3): 25–30.
28. Tsai YI, Hsieh L-Y, Ma Y-C, Kuo S-C. A novel method for determination of low molecular weight dicarboxylic acids in background atmospheric aerosol using ion chromatography. Anal Chim Acta 2008;626(1): 78–88.
29. Jenke D, Sadain S, Nunez K, Byrne F. Performance characteristics of an ion chromatographic method for the quantitation of citrate and phosphate in pharmaceutical solutions. J Chromatogr Sci 2007;45(1):50–6.
30. Meng H-B, Wang T-R, Guo B-Y, Hashi Y, Guo C-X, Lin J-M. Simultaneous determination of inorganic anions and cations in explosive residues by ion chromatography. Talanta 2008;76(2):241–5.
31. Bommarito CR, Sturdevant AB, Szymanski DW. Analysis of forensic soil samples via high-performance liquid chromatography and ion chromatography. J Forensic Sci 2007;52(1):24–30.
32. Singh J, Elberling JA, Hemphill DG, Holmstrom J. The measurement of nitrite in adulterated urine samples by high-performance ion chromatography. J Anal Toxicol 1999;23(3):137–40.
33. Kaine LA, Heitkemper DT, Jackson DS, Wolnik KA. Use of ion chromatography for the verification of drug authenticity. J Chromatogr A 1994;671(1–2):303–8.
34. Suzuki S, Tsuchihashi H, Nakajima K, Matsushita A, Nagao T. Analyses of impurities in methamphetamine by inductively coupled plasma mass spectrometry and ion chromatography. J Chromatogr A 1988;437(1): 322–7.
35. Haddad PR, Nesterenko PN, Buchberger W. Recent developments and emerging directions in ion chromatography. J Chromatogr A 2008; 1184(1–2):456–73.
36. http://leda.lycaeum.org/?ID=8847 (accessed September 14, 2010).
37. Chappell JS. The non-equilibrium aqueous solution chemistry of gamma-hydroxybutyric acid. JCLIC 2002;12(4):20–7.

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