Application of Inductively Coupled Plasma Atomic Emission and Mass Spectrometry to Forensic Analysis of Sodium Gamma Hydroxy Butyrate and Ephedrine Hydrochloride*

Invited Lecture

KAREN A. WOLNIK, DOUGLAS T. HEITKEMPER, JOHN B. CROWE, BARBARA S. BARNES AND THOMAS W. BRUEGGEMEYER

National Forensic Chemistry Centre, US Food and Drug Administration, Cincinnati, OH 45221, USA

The identity and relative amounts of various elements in samples of two compounds, sodium gamma hydroxy butyrate (GHB) and ephedrine hydrochloride (ephedrine), have been used to compare items of evidence. GHB, unapproved for use in the United States, and ephedrine, used in the illicit manufacture of methamphetamine, are currently of interest to law enforcement authorities. In this paper, the analysis of the elemental impurities in GHB by inductively coupled plasma atomic emission spectrometry (ICP-AES) has been used to further the investigation of clandestine manufacturing sources. The following elements were detected in samples of GHB: Ba, Ca, Cd, Fe, K, Mg, Ni, P, Pb, Si, Sr, and Zn. Results were used to demonstrate an association between samples of unknown origin. Analysis of samples of ephedrine of known origin from legitimate manufacturers by ICP-mass spectrometry showed that Al, Ba, Mn, Pt, Rb, and Sr were the most useful for discriminating between sources and making direct comparisons. Interpretation of results with respect to the intended use of conclusions derived from those results is discussed.

Keywords: *Inductively coupled plasma atomic emission spectrometry; inductively coupled plasma mass spectrometry; ephedrine hydrochloride; gamma hydroxy butyrate; sodium oxybate; forensic analysis*

Historically, forensic scientists have used the identity and relative amounts of various elements in samples, such as glass and paint, to differentiate or link items of evidence. In 1982 seven people in the Chicago area were poisoned by Tylenol capsules purposely tainted with potassium cyanide. In that instance, analysis by inductively coupled plasma atomic emission spectrometry (ICP-AES) of trace element contaminants in a relatively pure chemical, KCN, was used for making comparisons of suspect samples.[1] This technique has been extended to include ultratrace analysis by ICP-mass spectrometry (MS) and has been applied to a number of diverse forensic samples.[2–7]

ICP-AES and ICP-MS analysis of forensic drug samples, including methamphetamine, cocaine, heroin and legitimate bulk pharmaceuticals, has been described.[8–11] Elemental analysis of drug substances has the potential for providing information regarding geographic origin, synthetic route, and type of refining treatment used in addition to its usefulness in direct

sample comparison analyses. Comparative chemical analysis of cocaine samples using gas chromatography for the detection and quantification of manufacturing impurities has been used successfully in criminal prosecutions.[12,13]

Elemental analysis by ICP-AES and ICP-MS is applied here to two drugs, sodium gamma hydroxy butyrate (GHB) and (−)-ephedrine hydrochloride (ephedrine), currently of interest to law enforcement authorities. The relative amounts and identity of the trace elements present in these substances are evaluated for the ability to discriminate and classify various samples.

GHB, or sodium oxybate, is recognized as a hypnotic, an adjunct to anesthesia;[14] however, GHB has not been approved for use in the United States. Consequently, the sale and distribution of this drug is illegal. Nevertheless, GHB has been promoted as an ergogenic aid (a substance that increases some aspect of muscle performance) and is marketed to athletes, bodybuilders, and others. It is believed that GHB increases the secretion of natural growth hormone and/or that GHB will improve the quality of sleep and produce a 'euphoric' effect.[15] Use of GHB has caused serious illness in a number of people with symptoms including respiratory problems, seizures and coma.[16] According to popular literature targeted at users of ergogenic aids, sodium oxybate can be easily prepared by the alkaline hydrolysis of gamma-butyrolactone, a fairly common industrial solvent.[17]

Ephedrine, a stimulant less potent than methamphetamine, is often used in the illicit manufacture of methamphetamine, a controlled substance. Ephedrine is available in approved products over-the-counter in the United States. Unapproved products containing ephedrine can be purchased on the black market. In some instances, ephedrine is obtained from finished dosage form products. In other cases, bulk ephedrine is diverted from legitimate use to serve as the precursor.

EXPERIMENTAL

Reagents and Standards

The water used was distilled and de-ionized (DDW) (18 MΩ cm, Millipore). The nitric acid used was Baker, Instra-Analyzed (Phillipsburg, NJ, USA) for ICP-AES studies and GFS Chemicals (Columbus, OH, USA) Double Distilled for ICP-MS studies. Plasma-grade reagent standards (1000 μg ml^{-1}) were used in the preparation of all standard solutions (Spex Industries, Edison Park, NJ, USA and Inorganic Ventures, Toms River, NJ, USA).

Samples and Preparation

Samples of GHB were obtained as investigative samples and were prepared simply by adding 15 ml of 2% nitric acid to 0.5 g of powder in an acid-washed 30 ml high density polyethylene (HDPE) bottle. The mixture was shaken until all GHB dissolved.

Samples of ephedrine were collected by another government agency from two different sources and will be described here as 'Investigative' samples 1 and 2. Samples were also obtained from the designated manufacturer's reserve and are referred to here as 'Known' samples 1–4. In addition, four samples of ephedrine were obtained from commercial sources for use as comparison samples. These samples are described in this work as 'Comparison' samples 1–4.

Ephedrine samples were prepared by adding 1.25 g of 50% v/v nitric acid to 0.25 g of ephedrine. This mixture was then allowed to stand for 30 min prior to addition of an internal standard and dilution to a final mass of 10 g with DDW. Samples were prepared as described above in acid-washed 30 ml HDPE bottles and transferred to acid-washed autosampler tubes immediately preceding ICP-MS analysis.

Instrumentation

The simultaneous ICP-AES instrument used in this work was a Model 1160 Plasma Atomcomp (Thermo Jarrell Ash, Franklin, MA, USA). The instrument has an updated PC-based data acquisition and readout system. The nebulizer used was a fixed crossflow. The ICP-AES operating conditions are described in Table 1.

The ICP-MS instrument was a PlasmaQuad Model PQ2+ (Fisons, Winsford, Cheshire, UK) equipped with a Gilson Model 222 autosampler (Middleton, WI, USA). A Meinhard concentric nebulizer and Scott-type spray chamber cooled to 5 °C were used. The ICP-MS was operated in the scanning data acquisition mode. Operating conditions are listed in Table 2. The following isotopes were used for quantification: ^{27}Al, ^{55}Mn, ^{85}Rb, ^{88}Sr, ^{138}Ba and ^{195}Pt.

Table 1 ICP-AES operating conditions

Forward power/kW	0.95
Reflected power/W	<5
Outer gas flow/l min^{-1}	20
Intermediate gas flow/l min^{-1}	0.75
Injector gas flow/l min^{-1}	0.75
Solution delivery rate/ml min^{-1}	1.5
Viewing height/mm	15
Background correction/nm	±0.03

Table 2 ICP-MS operating conditions

Forward power/kW	1.35
Reflected power/W	<5
Outer gas flow/l min^{-1}	14
Intermediate gas flow/l min^{-1}	1.0
Injector gas flow/l min^{-1}	0.80
Solution delivery rate/ml min^{-1}	1
Analyser stage/mbar	2.1×10^{-6}
Intermediate stage/mbar	$<10^{-4}$
Expansion stage/mbar	1.5
Scanning parameters	
Pulse counting dwell/µs	320
Time/µs	320
Analogue dwell time	20
Channels per amu	3
No. of integrations	3
Skipped mass regions	12–23 and 28–42
Internal standard	10 ng ml^{-1} of In

RESULTS AND DISCUSSION

Sodium Gamma Hydroxy Butyrate

Evaluation of the elemental patterns that result from ICP-AES or ICP-MS analysis of a material depends on a number of factors in addition to the accuracy and precision of the analytical technique. The history of the samples being provided, the questions being asked about those samples, and the intended use of the answers to those questions must all be considered. In the early 1990s the Food and Drug Administration (FDA) was investigating widespread sales of GHB. Samples purchased undercover all across the country were referred to our laboratory for elemental analysis in the hope that the results would focus the investigation and provide information on production and distribution channels. In addition to direct comparison of various suspect samples, the investigators wished to know the number of illegal manufacturing sources of GHB. The certainty and statistical standards applied to 'exploratory data analysis', which is aimed at furthering an investigation, are less rigorous than required to provide 'proof' in a criminal prosecution.

Investigators located two illicit GHB manufacturing sites and samples were collected from each. Results obtained by ICP-AES for the replicate analyses of these 'known' samples are presented in Table 3. Samples from one of the manufacturing sites (Site A) consisted of two 'in process' portions of GHB (A-1 and A-2) and portions taken from 5 filled drums found at the site (A-3 through A-7). The comparison between the 'in process' and drum samples was significant since the possibility existed that the drum samples had been manufactured elsewhere. The sample obtained from the other site (Site B in Table 3) was a single sample of limited quantity, <600 mg. The results shown in Table 3 illustrate the similarities between the 'in process' samples and the drum samples. Note that concentrations have not been corrected for moisture. Results for drum A-3 are somewhat out of line, particularly for calcium, magnesium and strontium, but are much more closely aligned with Site A than Site B. The obvious differences in the levels of various elements, notably cadmium, lead, calcium, magnesium and strontium, found in the samples from the two sites, indicate the potential for discrimination between these two sources based on elemental analysis.

Other GHB samples purchased 'undercover' were compared with these samples of known origin. Plots of element X versus element Y were useful for facilitating comparisons of large numbers of results. Several suspect samples showed definite similarities to the 'known' samples while others tended to group together but were different from the 'known' samples. Examples of element versus element plots are shown in Fig. 1(a)–(c) for a set of 20 suspect samples (including the Site A drum samples) referred to our laboratory and analysed over a span of several months. Three replicate weighings were analysed for each suspect sample, and each sample is therefore represented on the plot by three points. Individual results for the known samples from Site A are symbolized by a star and from Site B by an asterisk. The drum samples and some suspects are symbolized with a circle while other suspects are symbolized with triangles or squares to permit comparison of one plot with another. A plot of calcium versus cadmium [Fig. 1(a)] served to separate the set into two groups which coincided with the two known manufacturing sites and corresponded to high versus low or non-detectable levels of these two elements. Strontium versus lead [Fig. 1(b)], and iron versus barium [Fig. 1(c)] further divided the set into three groups. It can be inferred that the suspect samples represented by squares were manufactured at Site B, and that the samples represented by circles are associated with Site A. The suspect samples symbolized by triangles appear to be distinct from the other groups. Other element-by-element plots either corroborated

Table 3 Average concentration ($\mu g\ g^{-1}$) in GHB samples: $n=3$, except for B-1 when $n=2$

Site No.	Ba	Ca	Cd	Fe	Mg	P	Pb	Si	Sr	Zn
A-1	0.165	139	≤0.073	2.49	11.2	≤1.8	≤1.4	27.5	1.56	0.262
A-2	0.160	148	≤0.073	2.64	11.7	≤1.8	≤1.4	27.3	1.67	0.280
A-3	0.143	76.6	≤0.073	3.14	6.10	1.9	≤1.4	24.4	0.761	0.407
A-4	0.195	149	≤0.073	2.96	11.4	≤1.8	≤1.4	26.6	1.55	0.914
A-5	0.234	200	≤0.073	3.56	14.4	≤1.8	≤1.4	29.8	2.24	0.508
A-6	0.195	154	≤0.073	2.15	11.9	≤1.8	≤1.4	27.9	1.71	0.822
A-7	0.222	200	≤0.073	3.59	14.3	≤1.8	≤1.4	28.8	2.23	0.665
B-1	0.136	7.82	1.94	8.08	≤1.1	3.26	3.58	16.9	0.140	0.937
Detection limits*	0.034	0.35	0.073	0.68	1.1	1.8	1.4	1.7	0.037	0.082

* Calculated as 3 times the standard deviation of 9 method blanks multiplied by the dilution factor, Ni and K were not detected in these samples.
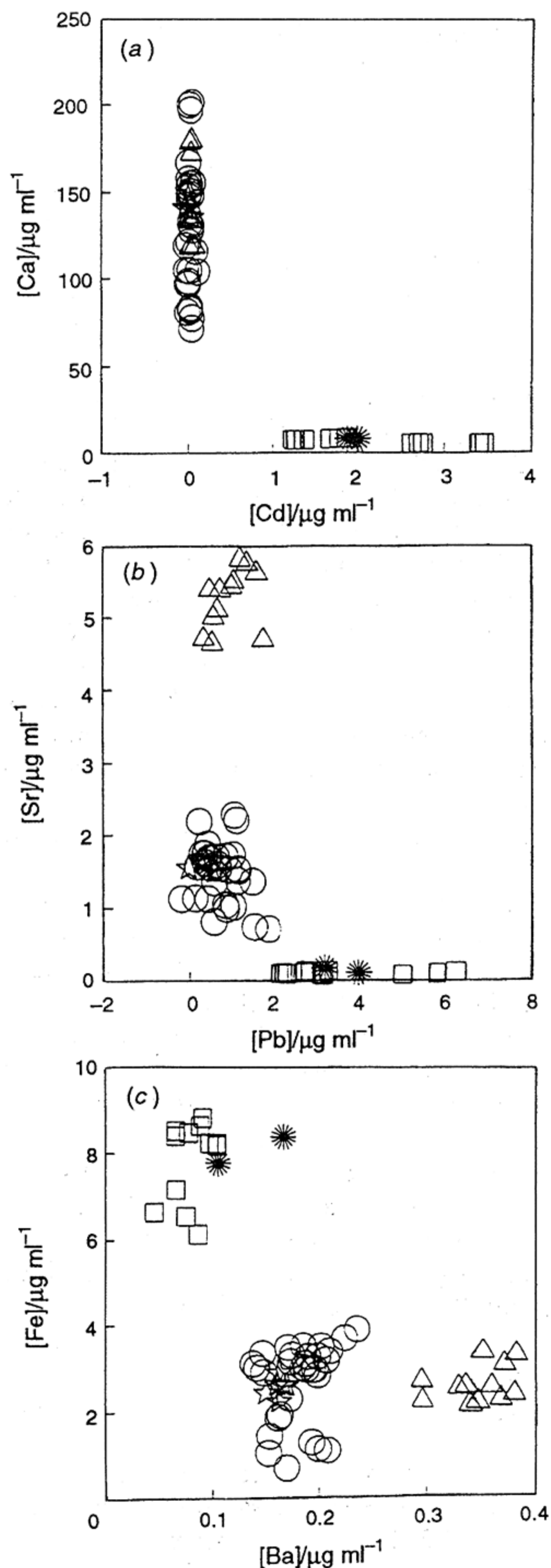

Fig. 1 Element *versus* element plots for GHB samples. ☆ Site A, ✳ Site B, △ Suspects, ○ Suspects, □ Suspects (see text for further details)

these three groupings or displayed points which were insufficiently resolved to aid in evaluation.

In total more than 100 samples of GHB were eventually analysed using ICP-AES. The following elements were detected in various samples: Ba, Ca, Cd, Fe, K, Mg, Ni, P, Pb, Si, Sr, and Zn. Analysis of GHB purchased from legitimate chemical distributors showed much lower levels of contamination with only a few elements above the detection limit. Comparison of elemental results for suspect and known samples and between suspect samples provided information useful to investigators. This information served as a basis for testing knowledge obtained from labels and various documents, for corroborating statements made by informants and for linking manufacture and distribution of GHB from coast to coast. However, we were unable to provide a definitive answer to the question of the number of sources of manufacture since the cause(s) for the observed variations in elemental results which led to the sample groupings could not be defined. The differences could represent different batches of production, different manufacturing sites, or different histories, *i.e.*, different sources of contamination.

(−)-Ephedrine Hydrochloride

The results for the ephedrine samples referred to our laboratory were intended for use in a criminal prosecution. The questions asked were well-defined; therefore, our approach to the elemental analysis of ephedrine was much more methodical.

Analytical method development

Preliminary ICP-MS scans were performed on several ephedrine samples to determine which elements might be useful in discriminating between ephedrine manufacturers. No quantification was performed in any of these preliminary experiments. Integrated area counts were compared for all scanned masses. Fourteen elements, including Na, Al, Mn, Rb, Sr, Mo, Sb, Ba, La, Ce, Gd, Dy, Pt and Pb, were selected for further study.

Most of the ephedrine samples were analysed quantitatively on two separate occasions. Seven of the original fourteen elements were found to provide a significant ability to differentiate various ephedrine samples (Na, Al, Mn, Rb, Sr, Ba, and Pt); however, only six of the seven provided reproducible values from the day-to-day runs. The within-day reproducibility for sodium was acceptable; however, the day-to-day reproducibility was poor. It is speculated that this was due to a problem with the cross calibration between the pulse counting and analogue detector modes of the ICP-MS. Higher levels of sodium and aluminium were found in some samples and required detection in the analogue mode. The remainder of the elements studied utilized only pulse counting detection.

Table 4 Ephedrine results

Sample	Al	Mn	Rb	Ba	Pt	Sr
			Element/ng g^{-1}			
Investigative 1						
Mean	622	123	10.5	264	3.2	25.9
SD ($n=8$)	109	16	1.3	48	0.4	3.7
Investigative 2						
Mean	1409	69.2	3.8	57.4	0.88	24.1
SD ($n=12$)	175	22.3	0.5	9.1	0.44	3.0
Known 1						
Mean	729	181	15.2	333	2.3	27.1
SD ($n=4$)	62*	19	1.4	76	0.3	3.3
Known 2						
Mean	638	199	11.5	261	6.5	33.5
SD ($n=6$)	55	9	1.2	57	1.7	4.5
Known 3						
Mean	1295	129	10.7	298	3.3	27.0
SD ($n=6$)	832	33	1.0	38	0.2	1.6
Known 4						
Mean	1278	75.5	3.8	64.1	2.8	23.4
SD ($n=6$)	154	21.8	0.2	8.0	0.9	3.0
Comparison 1						
Mean	563	74.6	1.2	trace	<DL	3.3
SD ($n=6$)	60	23.0	0.2			0.8
Comparison 2						
Mean	44199	75.7	1.1	trace	19.0	2.7
SD ($n=3$)	848	4.7	0.4		3.5	0.8
Comparison 3						
Mean	46574	79.3	1.2	8.1	17.9	2.5
SD ($n=3$)	3955	8.0	0.3	11.2	1.1	0.6
Comparison 4						
Mean	438	131	4.6	39.3	3.0	18.4
SD ($n=3$)	70	2	0.2	1.2	0.5	0.4

* $n=6$ for Al.

Elemental determinations

A summary of ICP-MS results for the six elements selected is shown in Table 4. The mean concentrations for the Investigative and Known samples are derived from at least three replicate weighings on each of two separate occasions. Comparison samples 2–4 were analysed in triplicate on only a single occasion. The reproducibility of sample determinations generally ranged from 2–35% with an average of about 15%. As expected, the relative standard deviations (RSDs) for concentrations closer to the detection limit, were significantly higher. The RSD for Al in Known sample 3 was 64%; however, the within-day reproducibilities were 8 and 28%. The reason for the difference from day-to-day is not known. Sample detection limits (DLs) were as follows: 7.2 ng g^{-1} Al, 3.2 ng g^{-1} Mn, 0.42 ng g^{-1} Rb, 0.42 ng g^{-1} Sr, 0.54 ng g^{-1} Ba, and 0.72 ng g^{-1} Pt. Detection limits were calculated as 3 times the standard deviation of 10 measurements of a method blank multiplied by a dilution factor of 40.

Comparison of results

Comparison of forensic samples for purposes of discriminating or classifying samples can be accomplished in a number of different ways including principal components analysis, k-nearest neighbours, and linear discriminant analysis. A recent review revealed 205 references dealing with chemical pattern recognition in the past two years.[18] However, when possible, methods which are simply applied and understood are preferred for forensic samples. As criteria for discrimination of glass samples analysed by ICP-AES, Hickman[3] simply compared the ranges (mean±2SD) of each of the elements determined for two samples. Samples were reported as indistinguishable if the ranges overlapped for every element. If the range for at least one element did not overlap, the samples were reported as distinguishable. Similarly, Zurhaar and
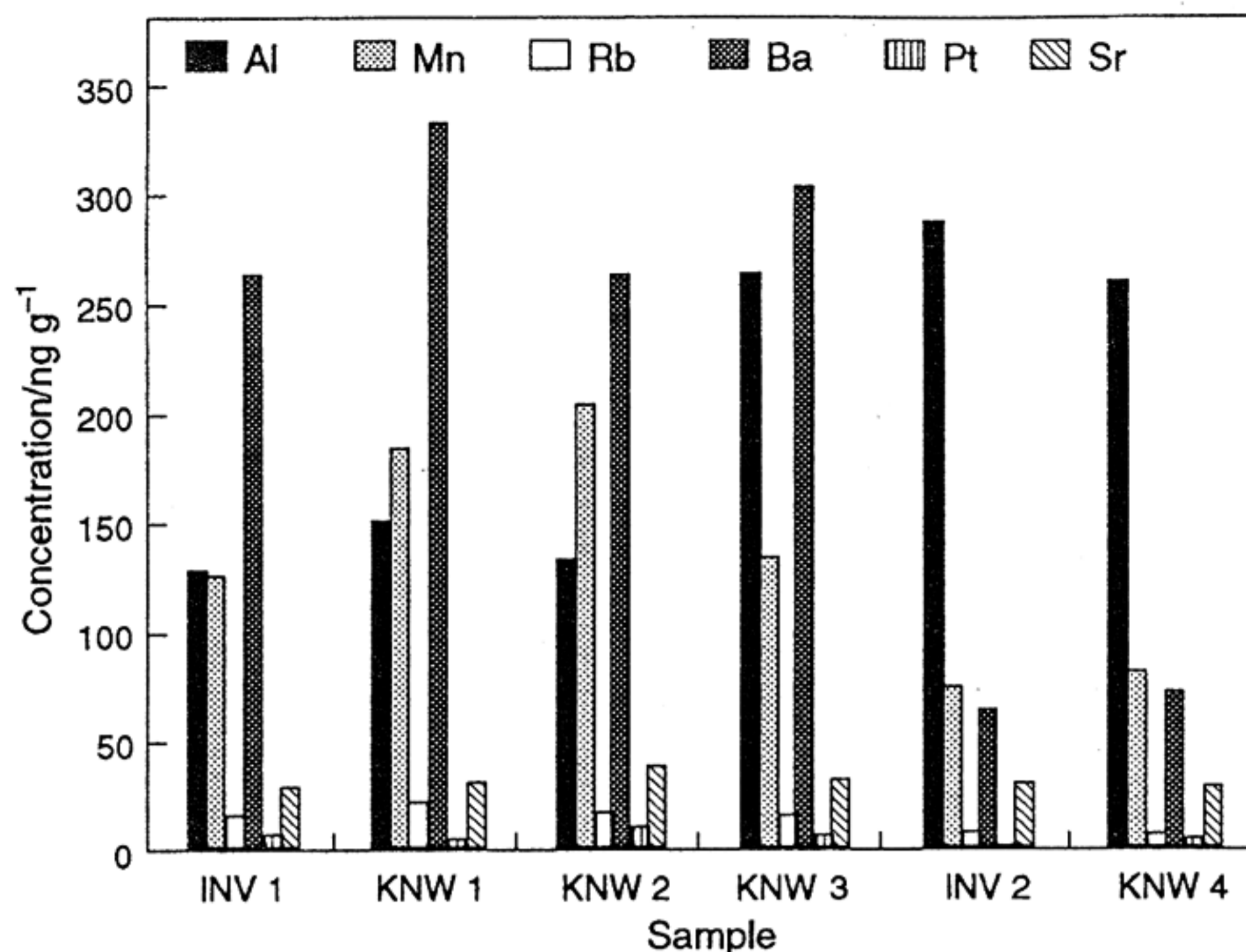

Fig. 2 Average concentration profiles for samples of (−)-ephedrine HCl for: Investigative samples (INV); and Known samples (KNW) (Al = Al ng g^{-1} ÷ 5)

Mullings[5] used the mean±3SD to discriminate glass samples analysed by ICP-MS.

The first question asked in the ephedrine investigation was, 'Are the two Investigative samples identical?'. Fig. 2 shows a comparison of the mean concentrations of Al, Mn, Rb, Ba, Pt and Sr for several samples including the two Investigative samples (INV1 and INV2). It should be noted here that the Al concentration has been divided by a factor of 5 for graphical purposes. The profiles for INV1 and INV2 appear to be different for Al, Mn, Rb, Ba and Pt. Using the discrimination criteria of Hickman[3], the two samples are distinguishable. The ranges for Al, Rb, Ba and Pt did not overlap. Significance testing was also used to compare the means for each element.[19] An uncertainty of the difference between the means was calculated using the appropriate Student t value and a pooled SD. This uncertainty is then compared with the measured difference between the means. At the 95% level of confidence, the Al, Mn, Rb, Ba and Pt mean concentrations are significantly different. Therefore, it was concluded that the two samples are distinguishable.

A number of analytical specification documents relating to ephedrine were obtained in conjunction with one of the Investigative samples of ephedrine. The second question regarded our ability to match the Investigative samples to the specification documents. In order to answer this question, reserve samples with lot numbers corresponding to those on the documents were obtained directly from the manufacturer. These are the aforementioned Known samples and make it possible to link an Investigative sample with information from the documents by direct comparative analysis.

Mean element concentrations for the Investigative samples and Known samples are shown in Fig. 2. Several observations were made on visual inspection of the element profiles. The profile for Investigative sample 1 has the same basic appearance as profiles for Known samples 1–3, which were produced at the same manufacturing plant. In addition, the profile for Investigative sample 2 appears very much like the profile for Known sample 4 which was manufactured at a different site than Known samples 1–3.

Significance testing was used to compare the mean element concentrations for Investigative sample 1 with those of Known samples 1, 2 and 3. For Known sample 3, only the mean Al concentration showed a significant difference with Investigative sample 1 at the 95% level of confidence. For Known samples 1 and 2, the mean concentrations were significantly different from Investigative sample 1 for three of the six elements.
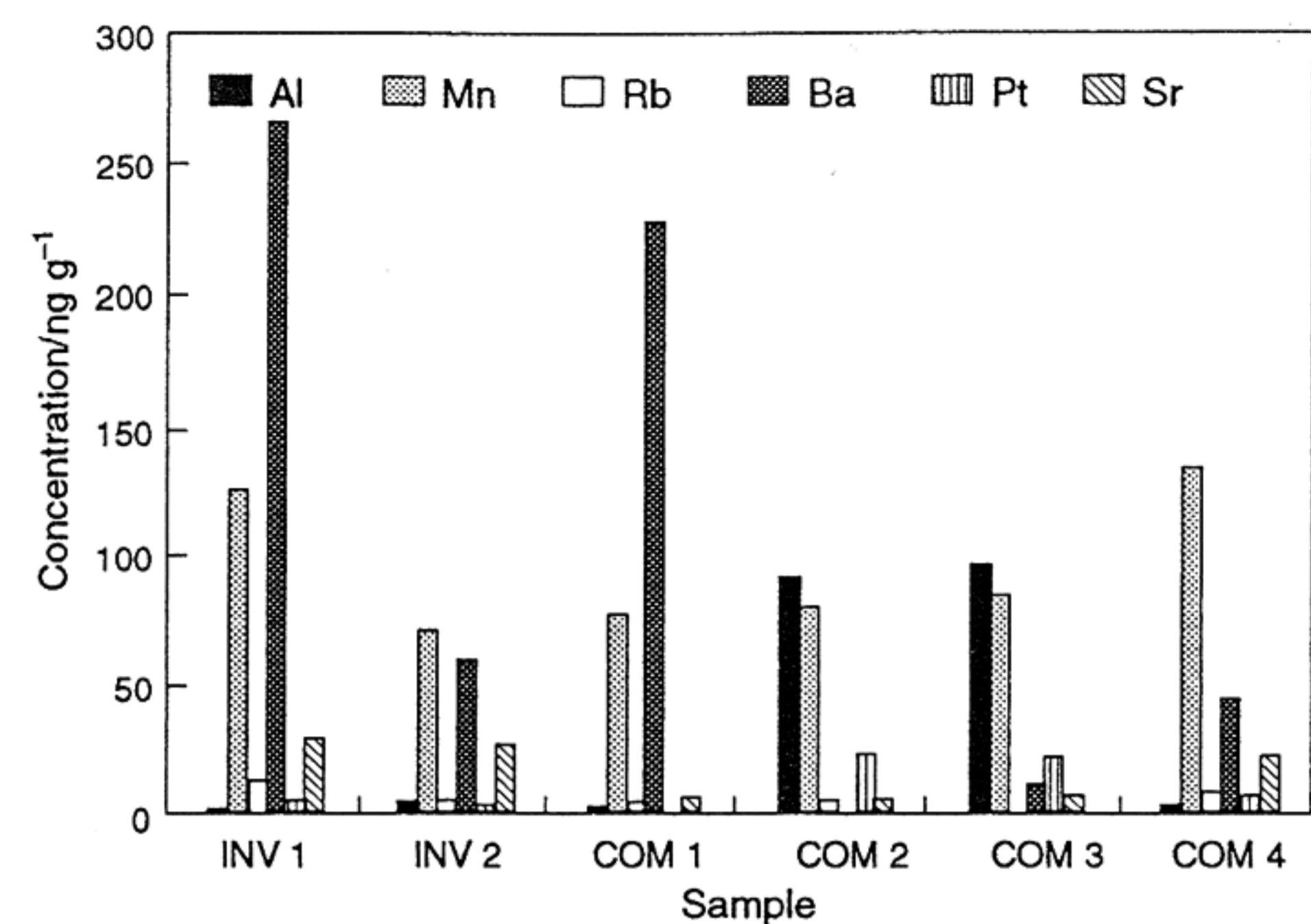
Fig. 3 Average concentration profiles for samples of (−)-ephedrine HCl for: Investigative samples (INV); and Comparison samples (COM) (Al = Al ng g^{-1} ÷ 500)

However, the ranges (mean ± 2SD) for each of the six elements determined in Known samples 1 and 3 overlap with those in Investigative sample 1. Thus, Known samples 1 and 3 and Investigative sample 1 are indistinguishable when using the range for discrimination.[3] It would seem highly likely that Investigative sample 1 and Known samples 1–3 are from a common manufacturing source.

The differences in mean element concentrations for Investigative sample 2 and Known sample 4 were also tested for significance as described above. At the 95% level of confidence, only the mean Pt concentration showed a significant difference. Nevertheless, if the ranges (mean ± 2SD) are compared as the measure of discrimination, the two samples are indistinguishable, i.e., the ranges for Al, Mn, Rb, Ba, Pt and Sr overlap. It would seem highly likely that Investigative sample 2 and Known sample 4 are from a common manufacturing source.

Four additional samples of ephedrine were obtained from commercial sources for comparison purposes (Comparison samples). Mean element concentrations for the Investigative and Comparison samples are shown in Fig. 3. Note that in this figure the Al concentration has been divided by a factor of 500 for graphical purposes. A number of observations were made regarding the element profiles. The profiles for Comparison samples 1, 2 and 3 are significantly different from the Investigative samples. Comparison sample 1 is distinguishable from the Investigative samples (mean ± 2SD). Comparison samples 2 and 3 are indistinguishable from one another but distinguishable from the Investigative samples. Distributors records, which were obtained after testing was completed,

showed the two samples (Comparison 2 and 3) originated from the same source. In addition, Comparison sample 4 shows some similarities to Investigative sample 2. The concentration ranges for Rb, Pt and Sr overlap in the two samples. Although the samples are distinguishable, distributors records showed that Comparison sample 4 and Known sample 4 were manufactured at the same site approximately 1 year apart from each other. The comparisons made using Fig. 3 would indicate that conclusions made earlier are valid and that it may be possible to go beyond simple direct comparison analysis and start to classify samples by manufacturing source, based on elemental analysis. This would of course require the analysis of many additional ephedrine lots which were produced over a specified time period.

REFERENCES

1 Wolnik, K. A., Fricke, F. L., Bonnin, E., Gaston, C. M., and Satzger, R. D., *Anal. Chem.*, 1984, **56**, 466A.
2 Koons, R., *Spectroscopy*, 1993, **8**, 16.
3 Hickman, D. A., *Forensic Sci. Int.*, 1983, **23**, 213.
4 Wolnik, K. A., Gaston, C. M., and Fricke, F. L., *J. Anal. At. Spectrom.*, 1989, **4**, 27–31.
5 Zurhaar, A., and Mullings, L., *J. Anal. At. Spectrom.*, 1990, **5**, 611.
6 Koons, R. D., Peters, C. A., and Rebbert, P. S., *J. Anal. At. Spectrom.*, 1991, **6**, 451.
7 Koons, R. D., Peters, C. A., and Merrill, R. A., *J. Forensic Sci.*, 1993, **38**, 302.
8 Suzuki, S., Tsuchihashi, H., Nakajima, K., Matsushita, A., and Nagao, T., *J. Chromatogr.*, 1988, **437**, 322.
9 Kishi, T., *J. Res. Natl. Bur. Stand.*, 1988, **93**, 469.
10 Violante, N., Quaglia, M. G., Lopez, A., and Caroli, S., *Microchem. J.*, 1992, **45**, 79.
11 Sheppard, B. S., Gaston, C. M., Barnes, B. S., and Wolnik, K. A., paper presented at the 1994 Winter Conference on Plasma Spectrochemistry, Abstract No. ThP26, San Diego, CA, January 1994.
12 Moore, J. M., Meyers, R. P., and Jimenez, M. D., *J. Forensic Sci.*, 1993, **38**, 1305.
13 Casale, J. F., and Waggoner, R. W., *J. Forensic Sci.*, 1991, **36**, 1312.
14 *The Merck Index, 11th Edition*, ed. Budavari, S., Merck and Co., Rahway, NJ, 1989, p. 1365.
15 Phillips, W. N., *Anabolic Reference Guide (6th Issue)*, 1991, Mile High Publishing, Golden, CO, pp. 36–37.
16 *FDA Quarterly Activities Report*, First Quarter, Fiscal Year 1991, Department of Health and Human Services, Public Health Service, Food and Drug Administration, p. 6.
17 Duchaine, D., *Underground Steroid Handbook for Men and Women, Update*, Daniel Duchaine, USA, 1992, pp. 45–48.
18 Brown, S. D., Blank, T. B., Sum, S. T., and Weyer, L. G., *Anal. Chem.*, 1994, **66**, 340R.
19 Taylor, J. K., *Quality Assurance of Chemical Measurements*, CRC Press, Boca Raton, FL, 1987, pp. 29–30.