

Microwave Assisted Digestion of Heroin Street Samples for Trace Metals Analysis by Inductively Coupled Plasma Mass Spectrometry

AURA LICSANDRU^{1*}, VERONICA NACEA², RICA BOSCEANU²

¹General Inspectorate of Romanian Police, Central Laboratory for Drug Analysis and Profiling, 13-15 Stefan cel Mare Blvd., 020123, Bucharest, Romania

²University of Medicine and Pharmacy „Carol Davila”, Faculty of Pharmacy, Inorganic Chemistry Department, 6 Traian Vuia Str., 020956, Bucharest, Romania

The present paper describes the development of a microwave acid digestion procedure for street heroin samples in order to achieve sample dissolution prior the determination of major and trace elements employing inductively coupled plasma mass spectrometry (ICPMS). Powdered sample aliquots were dissolved with various acid mixtures and different microwave digestion processes were tested. The optimized acid mixture for digestion of heroin samples consisted of 6 ml HNO₃ and 2 ml H₂O₂, and the best digestion program had 30 minutes with two ramps of microwave power up to 550 W. The use of this condition results in analytical recoveries of major (Al and Mg) and trace (Cu and Co) elements in the range between 92% and 104% with RSD% of less than 10%.

Keywords: heroin, microwave digestion, trace elements, ICP-MS, recovery

Heroin use, particularly injecting the drug, still accounts for the greatest share of morbidity and mortality related to drug use in the European Union [1]. Street heroin appears usually like a fine powder coloured from beige to brown, and having a diacetylmorphine content less than 60%. Dealers add a wide variety of cutting agents to the active substance, which is genuinely shadowed by a cocktail of related products and impurities resulting from the manufacturing process [2-5].

The comparative analysis of two or more heroin samples is frequently required with the view of assisting law enforcement authorities in the fight against illicit trafficking of this dangerous drug. A chemical drug profile includes information about the balance between the major (diacetylmorphine) and minor (acetylcodein, monoacetylmorphine, noscapine, papaverine) compounds in the sample [6], the fabrication process (the solvent residues), the interferences of street-level distribution network (the cutting agents: adulterants and diluents) and inorganic impurities (minerals absorbed by the plant of origin, metals left by the catalysts used in the process of fabrication of synthetic drugs etc.).

The inorganic impurities generally originate in various sources or stages, i.e. raw materials, reagents, solvents, reaction vessels etc. In the case of heroin samples, the main sources of trace metals are the growth environment of *Papaver Somniferum* (nutrient solutions or soils) and the process of morphine extraction from dried poppy latex, followed by the synthesis of diacetylmorphine.

A number of studies have examined the possibility of determination of trace metals from seized street heroin samples [7-10] and also from poppy seeds and capsules [11-13], poppy straw [14] and opium [15,16] using different analytical tools such as ETAAS [7], AAS [8, 11], GF-AAS [12], ICP-OES [9, 16], ICP-AES [7, 13], ICP-MS [10, 15] or XRD by SEM/EDS [14]. Most of the papers were optimistic about the potential use of inorganic impurities for the purpose of sample comparison and/or origin determination.

Since 1980, ICPMS has emerged as a powerful technique in the area of elemental analysis. It combines two well-known analytical tools, inductively coupled plasma (ICP) and mass spectrometry (MS) and offers extremely low detection limits which range up to parts per trillion, ppt. Moreover, these detection limits are broadly achieved for almost all the elements across the Periodic Table. Rapid multielement capability, simplicity in spectra, high accuracy and precision and the ability to measure specific isotopes are some major advantages of ICPMS over other analytical methods [15].

Sample preparation is a critical step in trace metal analysis and microwave digestion is one of the standard sample preparation procedure for elemental determination in analytical chemistry. The samples are heated in closed vessels together with a suitable acid solution to high temperatures so that they are completely degraded and dissolved. Increased rate and greater reproducibility of chemical reactions, high efficiency of the process and superior yields compared to conventional heating methods are some of the great advantages of microwave assisted heating [17].

There are little information about the use of closed vessel microwave digestion technique for drugs of abuse dissolution [18-20] and there is no available published method for the microwave digestion of heroin. The aim of the study is the development of an efficient microwave-assisted digestion method of heroin street samples, in order to bring them in a suitable form for elemental analysis by ICPMS. The accuracy of the optimized method is demonstrated by recovery data observed for spiked real samples.

Experimental part

Reagents and samples

Nitric acid ultrapur 60% (Merck) and hydrogen peroxide p.a. 30% (Fluka) were used as oxidizers.

Multi-element calibration Standard 3 (matrix per volume: 5% HNO₃ per 100 ml) and single element standard solutions

* email: aura_licsandru@yahoo.com; Tel.: 0742285994

Step	Program 1			Program 2			Program 3		
	Power (W)	Ramp (min)	Hold (min)	Power (W)	Ramp (min)	Hold (min)	Power (W)	Ramp (min)	Hold (min)
1	400	5	5	400	10	5	400	10	5
2	600	5	10	550	10	5	550	5	10
3	0	0	10	0	0	10	0	0	10

Table 1
MICROWAVE DIGESTION
PROGRAMS FOR THE SAMPLE
PREPARATION

(Aluminium, Cobalt, Copper, Magnesium 1000 ppm/2% HNO₃, 100mL and Yttrium 10 ppm/2% HNO₃, 100mL) from Perkin Elmer were used for calibration, sample fortification and internal standardization. Different solutions were prepared or diluted with double deionised water (18.2 MΩcm⁻¹ conductivity) delivered by a Milli-Q Millipore water system.

Ten heroin samples from larger seizures received for analysis at Central Laboratory for Drug Analysis and Profiling were subject to experimental investigation. Weight determinations were carried out using a CP224S-0CE Sartorius electronic analytical balance.

Microwave digestion

Microwave digestion was carried out in a Multiwave 3000 with high performance Rotor 8SXQ80 (80cm³ Quartz vessels, 120 bar) - Anton Paar GmbH, Graz. A hydraulic system supplies the rotor in order to measure pressure in every position, while an IR-sensor determines external temperature of the vessels.

Plastic and glass-ware were cleaned by soaking in 10% HNO₃ over night. They were rinsed before use with distilled and ultrapure water.

Five digestion methods were developed in a chronological time. Basically they differed in the type of acid mixtures (HNO₃ or HNO₃ and H₂O₂) and the microwave temperature programs used and presented in table 1.

Heroin samples (approximately 260 mg) were weighed directly in quartz vessel by transferring them precisely on the vessel bottom. For the optimized method 6 mL aliquot of nitric acid 65% and 2 ml of hydrogen peroxide 30% was added to each vessel, which were gently swirled to ensure all the powder is covered in the oxidizing mixture and then allowed to decompose at room temperature. After at least half an hour (a short pre-reaction time), samples were capped and subject to closed-vessel microwave digestion.

Four quality assurance samples were prepared: the blank, the spiked blank, the non-spiked samples and the spiked samples. At the end of the digestion procedure, samples were quantitatively transferred into clean 50 mL Sarstedt tubes and diluted to volume with ultrapure water prior to ICPMS analysis. Some of the final solutions were clear, colorless, indistinguishable from water and others had a very pale yellow color which could be observed when holding the volumetric flask up to the light.

Sample analysis

The determination of trace elements in heroin samples was performed on a Perkin Elmer ELAN DRC-e ICP-MS quadrupole spectrometer, equipped with a double-pass spray chamber (Scott-type) and a cross-flow nebulizer, both served as sample introduction device. A peristaltic pump was used for sample uptake. Daily performance and calibration experiments were performed to check adequate instrumental performance. The operating parameters of the instrument are detailed in table 2 and the Quantitative method in the Elan instrument control software was used. The detector was employed either in Analog or Dual mode of operation, depending on the level of metals concentration in the heroin samples.

The calibration and optimization of the instrument were done using a calibration standard solution containing 1 ppb of Mg, Al, Cr, Mn, Cu, Rh, Th, Cd, In, Ce, Pb in 1% HNO₃ (Elan 6100 Setup/Stability/Masscal Solution from Perkin Elmer) in order to obtain the maximum Mg intensity for optimal counting statistics (>5000 cps), as well as oxide/double charge ion levels lower than 3%.

Working standard solutions were prepared through the serial dilution method on the same multi-element stock standard (Standard 3). Yttrium was used as internal standard for all elements, with final concentration of 100 ppb. It was spiked into each blank or sample solution and used to account for fluctuations in instrument responses.

Elements analyzed with ICP-MS in the present study were Na, Mg, Al, Mn, Sr, Ba, Co, Cu, Cr, Ni, V and four analytes were selected to check the accuracy of the mineralization process: Mg, Al (two of the major elements in illicit heroin), Cu and Co (two of the trace elements in illicit heroin). We obtained the following calibration equations:

$$y(\text{Mg}) = 0,00269358x(\text{Mg}) + 0,00422983, \quad \text{with } r(\text{Mg}) = 0,999981$$

$$y(\text{Al}) = 0,00354177x(\text{Al}) + 0,000283076, \quad \text{with } r(\text{Al}) = 0,999969$$

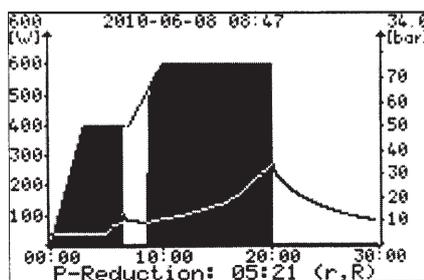
$$y(\text{Cu}) = 0,00260546x(\text{Cu}) - 0,0010632, \quad \text{with } r(\text{Cu}) = 0,999955$$

$$y(\text{Co}) = 0,00531556x(\text{Co}) - 0,000570547, \quad \text{with } r(\text{Co}) = 0,999994$$

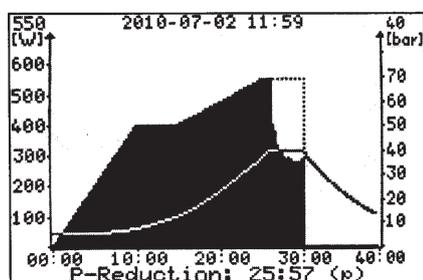
The values of the correlation coefficients for the calibration curves of all analytes were included in the interval 0.999785– 0.999996, better than minimum value of 0.990 admitted in international regulations concerning this performance criterion [21].

Inductively coupled plasma	
RF power for ICP	1300 - 1350 W
Plasma gas flow	15 L/min
Auxiliary gas flow	1.20 L/min
Nebulizer gas flow	0.95 L/min
Data acquisition	
Scanning mode	Peak Hopping
Sweeps	30
Dwell time	50 ms
Replicates	3
Calibration type	External calibration

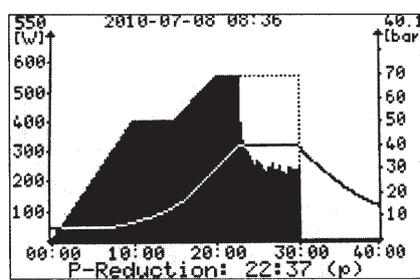
Table 2
ICP-MS OPERATING CONDITIONS



(a)



(b)



(c)

Fig. 1 Power and pressure curves for microwave digestion of heroin samples during optimization; (a) Program 1; (b) Program 2; (c) Program 3

Results and discussion

Determination of the experimental conditions for microwave digestion of heroin samples

Heating process in the microwave field depends on various factors (i.e. the nature of the sample, its weight, etc). Two samples will exhibit the same behaviour only in rare instances. Nevertheless, there is no certified reference material (CRM) with an identical matrix as the illicit heroin powder. Consequently, street samples were considered in order to optimize the microwave digestion process.

High temperature is necessary to facilitate fast and complete reactions, which afterwards result in degradation and dissolution of the samples. In theory, temperature is the only parameter which is actively influenced by the microwave radiation, while pressure simply being a by-product of the reaction at elevated temperatures. Furthermore, it is known that in closed systems, concentrate nitric acid has sufficiently high oxidation potential at high temperature and readily forms soluble nitrates with most metals (1). The addition of hydrogen peroxide to the digestion mixture will certainly increase the oxidation power of nitric acid during mineralization and enhance the attack of organic matter (2).



For these reasons, the digestions were carried out using either concentrate nitric acid alone or a mixture of concentrate nitric acid with hydrogen peroxide. In an attempt to reduce other possible sources of contamination, the number of reagents was limited by avoiding additional use of ultrapure water.

As the microwave digestion system is not equipped with an internal sensor for a precise measurement of the temperature and pressure inside the high-pressure vessels, further methods based on a power profile were consequently set up. The main goal was to ensure apx. 180°C IR temperature for samples' quartz tube, which approximate a temperature of 200-210°C inside the vessel [1]. In this context, digestion of the heroin samples results in a good and complete mineralization.

By increasing the magnetron power from 0 to 400W and later to 600W through short power ramps (Program 1), a final 180°C IR temperature could not be rapidly achieved because the spontaneous reactions that took place led to a fast pressure build-up. After 5min21s, when system had come to the pressure increase rate limit, the microwave power was switched off (fig. 1). To the contrary, a longer heating ramp allows a gradual raising temperature of samples and results in a controllable increase in pressure. So, Program 2 and Program 3 (table 1) were then developed as valuable alternatives to Program 1 in order to complete a safe and efficient digestion.

Figure 2 displays the temperature vs. pressure profile of the same heroin sample (260 mg) with 6 mL HNO₃ and 2 mL H₂O₂ during digestion paths of Program 2 and Program 3. After mineralization we evaluated the efficiency of both programs and we observed that recoveries of Al, Mg, Cu and Co spiked into the sample labelled Heroin 2 were superior for Program 3 (fig. 3), even though maximum IR temperature recorded in Program 2 was higher than in Program 3 (over 180°C in both cases, anyway).

Although it is known that H₂O₂ increases the oxidation potential of HNO₃, it could bring also an additional risk for sample contamination. That's why the influence of H₂O₂ over HNO₃ during the microwave digestion of the same heroin sample with Program 3 was investigated (fig. 4). In the absence of hydrogen peroxide the limit pressure of 40 bar was exceeded earlier and the final temperature outside the vessels didn't reach 180°C. On the contrary, when hydrogen peroxide was used, produced during digestion were re-oxidized into the pressure inside the reaction vessels was lower and so, the magnetron power was reduced later. These resulted in higher external and implicit internal temperatures. Moreover, better recoveries were obtained with HNO₃ and H₂O₂ as digestion mixture.

IR-graphs recorded over optimization process by Programs 1-3 (fig. 5) shows that:

- needed IR temperature (apx. 180°C) were reached during mineralization in all cases;
- a certain degree of uniformity has been noted in all vessels when Program 1 and 2 were involved (fig. 5: a, b), while Program 3 creates a quite similar behaviour for all samples digested in a run, suggesting the optimized procedure (fig. 5: c).

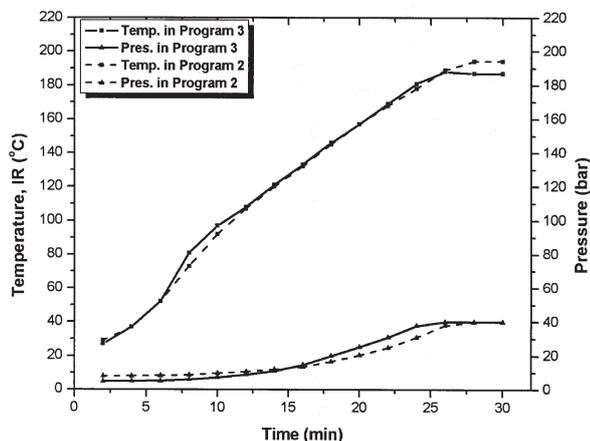


Fig. 2. IR temperature curves (---, ·····) and pressure curves (—▲—, ···▲···) obtained during the microwave digestion of heroin, by Program 2 (····, ···▲···) and Program 3 (—▲—, —▲—).

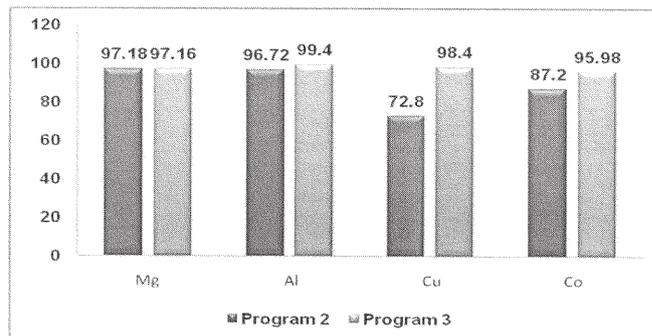


Fig. 3. Percent recoveries of Mg, Al, Cu and Co from Heroin 2 using microwave-assisted digestion conditions of Program 2 and Program 3

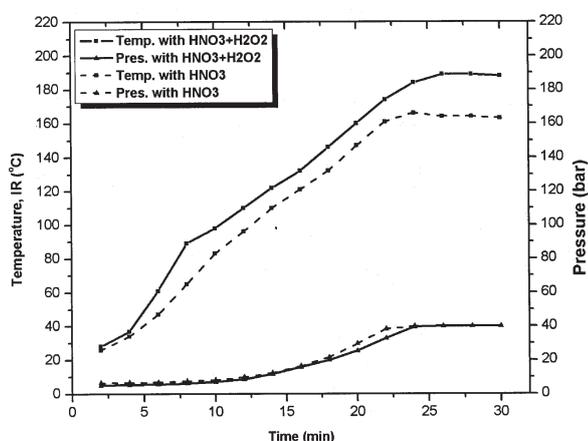
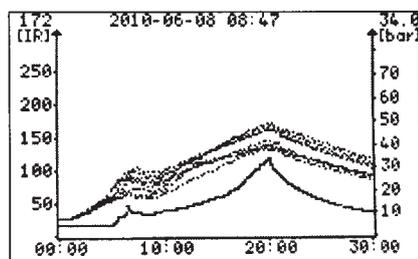
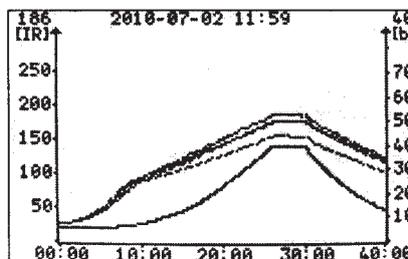


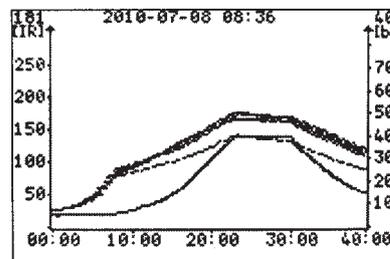
Fig. 4. IR temperature curves (---, ·····) and pressure curves (—▲—, ···▲···) during the microwave digestion of heroin, using HNO₃ (····, ···▲···) and HNO₃ + H₂O₂ (—▲—, —▲—).



(a)



(b)



(c)

Fig. 5. IR temperature and pressure curves for microwave digestion of heroin samples during optimization; (a) Program 1; (b) Program 2; (c) Program 3

Recoveries

In the lack of any CRM able to mimic organic matrix of heroin, the digestion processes corresponding to Program 2 and Program 3 were evaluated through recovery assays. Before mineralization, four heroin samples were spiked at 2 level of concentration. At the end, they were analyzed in 3 replicates. Fortification was performed with Mg, Al, Cu, Co and the recovery percentage was calculated as follows:

$$\%R = \frac{\text{metals content of the spiked samples} - \text{metals content of the non-spiked samples}}{\text{the expected metals content}} \times 100 \quad (3)$$

As seen from values presented in table 3, Program 3 is more effective than Program 2, the difference being particularly relevant for metals at lower concentrations (Cu, Co).

Table 3
PERCENTAGE RECOVERY IN SPIKED HEROIN SAMPLES DIGESTED WITH
PROGRAM 2 AND PROGRAM 3

Sample	Element	Spiked (ppb)	PROGRAM 2		PROGRAM 3	
			Found*±SD (ppb)	Recovery (%)	Found*±SD (ppb)	Recovery (%)
Heroin 1	Al	-	173.696±0.79	-	210.232±0.44	-
		300	462.656±0.18	96.32	496.843±0.81	95.53
		400	586.930±0.82	103.31	604.517±0.72	98.57
	Mg	-	562.362±0.48	-	608.333±0.37	-
		300	861.822±1.33	99.82	907.193±1.54	99.62
		400	980.242±1.43	104.47	1002.333±1.49	98.50
	Cu	-	1.656±0.01	-	1.886±0.01	-
		5	5.605±0.06	78.98	6.524±0.05	92.76
		10	10.281±0.22	85.95	11.688±0.07	98.02
	Co	-	0.181±0.01	-	0.108±0.01	-
		5	4.729±0.06	90.96	5.025±0.05	98.34
		10	10.381±0.05	102	10.029±0.05	99.21
Heroin 2	Al	-	492.583±1.10	-	492.639±0.91	-
		300	782.743±1.15	96.72	790.846±0.80	99.40
		400	978.503±0.42	121.48	895.362±0.63	100.68
	Mg	-	603.946±1.35	-	572.922±0.35	-
		300	895.486±0.82	97.18	864.407±0.77	97.16
		400	1012.546±2.60	102.15	978.404±1.2	101.37
	Cu	-	15.437±0.06	-	13.530±0.09	-
		5	19.127±0.25	72.80	18.450±0.05	98.40
		10	20.834±0.16	53.97	22.991±0.10	94.61
	Co	-	0.126±0.01	-	0.094±0.01	-
		5	4.487±0.02	87.20	4.893±0.04	95.98
		10	9.600±0.15	94.74	10.222±0.05	101.28
Heroin 3	Al	-	256.665±1.99	-	260.308±0.57	-
		300	564.915±1.29	102.75	561.778±0.69	100.49
		400	664.385±1.25	101.93	651.152±0.65	97.71
	Mg	-	769.941±2.41	-	824.773±0.94	-
		300	1075.491±1.64	101.85	1135.186±1.34	103.47
		400	1187.941±3.15	104.50	1239.654±1.82	103.72
	Cu	-	2.579±0.03	-	2.743±0.05	-
		5	5.999±0.18	68.40	7.605±0.03	97.24
		10	10.371±0.24	77.92	12.284±0.05	95.41
	Co	-	0.129±0.01	-	0.140±0.01	-
		5	4.595±0.02	89.32	4.837±0.03	93.94
		10	9.598±0.14	94.69	10.052±0.05	99.12
Heroin 4	Al	-	181.986±1.01	-	172.240±0.31	-
		300	474.276±0.71	97.43	471.707±0.52	99.82
		400	607.906±0.94	106.48	559.528±0.67	96.82
	Mg	-	651.913±1.31	-	660.633±0.81	-
		300	946.153±1.15	98.08	948.994±1.18	96.12
		400	1070.993±2.28	104.77	1072.042±2.20	102.85
	Cu	-	3.207±0.01	-	3.418±0.01	-
		5	7.004±0.06	75.95	8.107±0.04	93.78
		10	11.287±0.08	80.80	13.021±0.09	96.03
	Co	-	0.141±0.01	-	0.227±0.01	-
		5	4.717±0.03	91.52	5.190±0.04	99.26
		10	9.377±0.09	92.36	10.098±0.03	98.71

*Mean of three replicates

Conclusions

Nowadays, illicit drugs represent a major problem all over the world. Heroin street samples contain trace elements which may be identified and quantified using inductively coupled plasma mass spectrometry, an extremely sensitive and accurate analytical method. The presence of inorganic impurities in heroin samples differentiates them and can, at a later stage, disclose information about drug origin.

The heroin powder microwave acid mineralisation is the first and most important step preceding the determination of trace metals. The research aimed to improve and optimize the method used to achieve sample dissolution prior to elemental analysis by ICPMS.

As a standard procedure powders of street heroin were mineralized through different microwave digestion processes (Programs 1 to 3). Two reagent types (HNO₃, respectively HNO₃ + H₂O₂) were tested.

A combination between Program 3, described in Table 1, and a mixture of HNO₃:H₂O₂ = 6:2 (v/v), used for samples dissolution, allowed a recovery percentage of four elements

(Al, Mg, Cu, Co) in the range of 92%-104%, in compliance with most recent guidelines [22], with a precision of less than 10%. Thus, the microwave digestion method was optimized to a safe, precise and accurate tool for heroin analysis.

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