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Development of a slurry introduction method for multi-element analysis of antibiotics by inductively coupled plasma atomic emission spectrometry using various types of spray chamber and nebulizer configurations

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Abstract

A direct sample introduction inductively coupled plasma atomic emission spectrometric (ICP-AES) method, for multi-element analysis of powdered antibiotic drugs was developed using the slurry formation technique. The slurry of powdered sample is formed in dilute nitric acid solution in presence of Triton X-100 surfactant. Two different configurations of spray chamber and nebulizer were tested for direct aspiration of slurry into the plasma: (i) cyclonic spray chamber combined with babington-type nebulizer and (ii) scott-type double-pass spray chamber combined with cross-flow nebulizer. The latter configuration proved to be less tolerable to slurry aspiration. RF power generator, nebulizer argon gas flow rate, nebulizer sample uptake flow rate and slurry sample concentration were optimized. The sensitivity of the proposed method was compared to the corresponding sensitivity obtained from aqueous solutions for each analyte. The performance characteristics of the slurry aspiration method were evaluated against the complete acid-digestion method followed ICP-AES. Finally, the proposed method was applied to the analysis of commercial antibiotics.

Keywords: Antibiotics; Slurry; Inductively coupled plasma; Atomic emission spectrometry; Multi-element analysis; Nebulizer

1. Introduction

Antibiotic and antibacterial drugs in the form of syrups, liquids, tablets and powders are extensively used to treatment of various diseases and infections. The continuous increase of the use of antibiotics and other pharmaceuticals production and consumption leads to development of rapid and sensitive methods of determination of heavy metals and other contaminants which can be used in quality control projects and routine analysis. This is of increasing importance because of the toxic effect of many metallic ions in human life and the possibility of them to alter the efficiency of the drugs through formation of stable metal–drug complexes [1–3] and under certain conditions catalyze the degradation of the antibiotics [4,5]. The commercial antibiotic formulations usually contain the active pharmaceutical ingredients (APIs) and various organic and inorganic excipients. Residues of heavy metals is not likely to be found in the main synthetic pharmaceutical substance, however, when catalysts are used during certain steps of the synthetic procedure this risk should be tested [6]. If a specific metal catalyst is used and the synthetic processes are suspected to lead to the presence of residues of this metal, an element specific assay should be undertaken to determine the actual amounts of the residues. Also, it is possible to detect the presence of traces in the final product when a natural substance is used or when various excipients, diluting agents, natural flavors, etc. are included, without proper purification.

Taking into account the maximum allowable patient exposure to an element, certain limits of heavy metals concentration in pharmaceutical products can be specified. International pharmacopoeias recommend routine detection using heavy metals limit tests [7]. These tests are indicative for the overall quality of production, which includes sources of metal contamination, like manufacturing equipment and environment. The four equivalent test methods proposed in pharmacopoeias [7–9] are based on wet-acid sample extraction or dry ashing and ignition, precipitation at pH 3.5 (acetate buffer) of the colored metal sulphides

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using thioacetamide and subsequent visual comparison test for possible presence of metals higher than an equivalent limit concentration of a standard lead solution. The main disadvantages of the above method are the non-specific and non-sensitive determination of the analytes and also the time consuming digestion or ignition procedures.

For single element analysis, the atomic absorption spectrometry is commonly applied [10–13], or atomic emission spectrometry [14,15] after digestion of the pharmaceutical sample. The development of plasma atomization techniques has recently enabled the multi-element analysis of various matrices, down to the $\mu g\,l^{-1}$ concentration level. Inductively coupled plasma atomic emission spectrometry (ICP-AES) [16,17], and mass spectrometry (ICP-MS) [18–20] have been also reported for the analysis of drugs.

In spite of the fact that the multi-element determination is very important in the pharmaceutical industry, no plasma emission methods have been reported in the literature for multi-element analysis of antibiotic commercial drugs, as far as we know. ICP-AES could serve as a convenient multi-element technique for monitoring and screening analysis of such samples, because it offers the significant advantage of less costly instrumentation, as compared to other multi-element techniques.

The most common approach to analyze pharmaceutical samples by atomization techniques is the application of a preliminary wet or dry sample digestion step [11,14] and aspiration of the resulting solution into the nebulizer. Direct introduction of solid sample in the form of slurry is an alternative technique, which offers significant advantages such as eliminating time-consuming digestion steps and avoiding possible losses or contamination. Solid samples must be in powder form and aspirated in the form of slurry. Finally, simple sample dilution is another approach that can be applied to analyze water-soluble or miscible liquid substances, like syrups [17] and substances miscible with suitable organic solvents [21].

The aim of this work was to develop and optimize a method of direct aspiration of slurry into the nebulization system of inductively coupled plasma atomic emission spectrometer for quantitative multi-element analysis of powdered antibiotic formulations. The slurry is prepared by mixing the ground pharmaceutical tablets with dilute nitric acid and Triton X-100 solution. Two different configurations of nebulization systems were investigated: a cyclonic spray chamber combined to babington-type nebulizer (abbreviated, as Cyclonic/Babington, in the following text) and a Scott-type double-pass spray chamber combined with a cross-flow nebulizer (abbreviated as Scott/cross-flow). The optimum operating conditions of the ICP were examined. The maximum slurry concentration, which could be aspirated without extinguishing the plasma, was investigated for both the examined configurations. The performance characteristics of the proposed method were evaluated and compared to the results obtained after conventional acid-digestion of the antibiotic drugs. Finally, it was applied successfully to the analysis of a number of common commercial antibiotic drugs.

2. Experimental

2.1. Instrumentation

A Perkin-Elmer Optima 3100 XL axial viewing spectrometer was employed and used according to the operating conditions described in Table 1. The analytical wavelengths were set at the first and second sensitivity order spectral atomic (I) or ionic (II) lines of analytes listed in Table 2. A three-point background estimation procedure was applied in order to minimize possible spectral interferences. In case of extremely high concentration of Fe, which normally is not expected is such type of samples, the possible interference of the iron line on the nearby line of cobalt Co II 238.892 nm can be eliminated by using the inter-element correction procedure, which is an algorithm normally included in the operating software of the ICP instruments.

The plasma torch was consisted from alumina, which is sufficiently resistant to acidified solutions. A peristaltic pump was used to introduce the sample solutions into the ICP-AES at flow rates 1–3 ml min^{-1} and in the meantime to discard the wastes. Tygon type PVC peristaltic pump tubes (i.d. 0.030 in.) were used for sample delivery. Two nebulizer/spray-chamber configurations, as presented in Table 1, were examined in order to evaluate their applicability to handle high solid slurries and to estimate the different ICP conditions needed in each case. These two con-

Table 1
Operating conditions and instrumentation of the ICP-AES

RF generator	40 MHz, free-running
RF incident power	Optimized (1500 W)
Torch type	Fassel type
Injector, i.d.	Alumina, 2.0 mm
Viewing mode	Axial
Auxiliary argon flow rate	0.50 l min^{-1}
Nebulizer argon flow rate	0.80 l min^{-1} (optimized)
Plasma gas flow rate	15 l min^{-1}
Nebulization configuration	(i) Cyclonic spray chamber—Babington nebulizer (ii) Scott double-pass spray chamber—gem tip cross-flow nebulizer
Sample propulsion	Peristaltic pump, three channel
Sample uptake flow rate	3 ml min^{-1} (optimized)
Polychromator/resolution	Echelle/0.006 nm at 200 nm
Detector	Segmented-array charge-coupled (SCD)

Table 2
Spectral wavelengths used for ICP-AES determinations

Ag	I 328.068 nm	I 338.289 nm
Al	I 308.215 nm	I 237.313 nm
As	I 188.979 nm	I 193.696 nm
Ba	II 233.527 nm	II 230.424 nm
Be	I 313.107 nm	I 234.861 nm
Bi	I 223.061 nm	II 190.171 nm
Ca	II 317.933 nm	II 396.847 nm
Cd	II 214.440 nm	II 226.502 nm
Co	II 228.616 nm	II 238.892 nm
Cr	II 283.563 nm	II 284.325 nm
Cu	I 324.752 nm	II 224.700 nm
Fe	II 238.204 nm	II 239.562 nm
Ga	I 294.364 nm	I 209.134 nm
In	II 230.606 nm	I 325.609 nm
Mg	II 279.077 nm	II 280.271 nm
Mn	II 257.610 nm	II 259.372 nm
Ni	II 221.648 nm	II 232.003 nm
Pb	II 220.353 nm	I 217.000 nm
Pd	I 340.548 nm	I 363.470 nm
Se	I 196.026 nm	I 203.186 nm
Zn	I 213.857 nm	II 202.548 nm

(I) Atomic and (II) ionic lines.

figurations are the most commonly used and at least one of these is usually available in analytical laboratories equipped with ICP instrumentation.

A Perkin-Elmer 5100 flame atomic absorption spectrometer was employed for independent measurement of metal adsorption by the antibiotic formulations, using for each analyte the flame conditions recommended by the manufacturer (oxidizing flame for all analytes except Cr for which a reducing flame is needed). Simple aqueous calibration was employed, because all measurements referred to the aqueous supernatant solution. Fe, Mn, Cu, Cr, Co and Ni where determined at the following absorption spectral lines: 248.3 nm, 279.5 nm, 324.7 nm, 357.9 nm, 240.7 nm and 232.0 nm, respectively.

2.2. Reagents and solutions

Ultra pure water of Milli-Q quality (18.2 MΩ, Millipore, Bedford, USA) was used. All chemicals were of analytical reagent grade (pro analysi, p.a.) and were provided by Merck (Darmstadt, Germany). The reagents used for preparation of multi-element standards were of analytical grade, provided by Merck. Triton X-100 provided by Merck was used as surfactant reagent for slurry formation. A working standard solution containing 10 mg l^{-1} of all the above listed analytes was prepared by mixing suitable aliquots of a multi-element stock solution (Merck) containing Ag, Al, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, In, Mg, Mn, Ni, Pb, Zn, 1000 mg l^{-1} each, with four single-element stock standard solutions (containing 1000 mg l^{-1} of each of the following As, Be, Pd, Se, respectively) in 0.5 mol l^{-1} HNO_3 (Merck) and appropriate dilution. All solutions were stored in polyethylene bottles in the refrigerator. The above solution was further diluted, by using 0.5 mol l^{-1} HNO_3 as diluent, to obtain a series of lower concentration standards (0, 10, 50, 100, 200, and 500 μg l^{-1} for each analyte). The compatibility and storage stability of the above 21 elements multi-element standard was checked for a period of a month, and no precipitation or turbidity was observed. Finally, a calibration test of the stored multi-element standard against a freshly prepared one showed no analyte losses. Using the above described aqueous multi-element standards, six-point calibration curves were prepared. Also, the performance of standard addition procedure was examined by using the above aqueous standards as diluents for preparation of slurries. The slope of the calibration curves was used to estimate the sensitivity in all cases.

2.3. Procedure for slurry formation

Antibiotic samples in the form of tablets were collected from the market, ground and sieved. In all cases a portion of particle size fraction <100 μm was used for slurry formation. The tolerant slurry concentration was investigated by using suitable amounts of powdered sample, 0.5 mol l^{-1} HNO_3 as diluent solution and Triton (0.5%, v/v) as surfactant. The slurries were homogenized by stirring at 600 rpm and introduced during continuous stirring into the nebulization system using a peristaltic pump in appropriate flow rate. The nebulizer system and the inner tube (injector) of the torch were cleaned at least every hundred runs, with common rinsing solution (1% HNO_3) for ICP in order to avoid any trace carbon deposits. Alternatively, frequent aspiration of the diluent solution can be used.

2.4. Procedure for wet digestion of samples

Because there is no commercially available multi-element standard reference material of the same nature and matrix as the antibiotic powders the wet acid digestion procedure was used in order to evaluate the accuracy of the developed slurry formation method. The samples were completely acid digested in closed polytetrafluoroethylene (PTFE) vessels. An accurately weighed portion of the powdered sample (<100 μm), ca. 0.2 g, was weighed into PTFE vessel, with the subsequent addition of 5 ml of concentrated HNO_3 (65%, m/m) and 1 ml of H_2O_2 (30%, m/m). The vessels were closed, placed into a steel pressurized bomb and heated on a hot plate up to 130 °C for 2 h. The final digest was diluted to 25 ml in volumetric flask with 0.5 mol l^{-1} HNO_3. Blanks of the whole method were prepared following exactly the same acid digestion procedure. The digested solutions were analyzed by ICP-AES against acidified aqueous standards. All glassware and digestion vessels were soaked in freshly prepared 10% (v/v) HNO_3 for 24 h, and finally washed five times with Milli-Q quality water.

3. Results and discussion

Univariate optimization method was carried out for chemical and instrumental parameters using the two types of nebulization configurations, Cyclonic/Babington and Scott/cross-flow. Due to low concentrations of some trace elements in the investigated samples, the optimization was carried out using amoxicillin-containing slurries spiked with 200 μg l^{-1} of each analyte. The net signal was calculated after subtraction from the

recorded signal of the intensity of the not spiked slurry sample. Finally, the optimum spectral line for each analyte was selected taking into account the higher sensitivity, as it is described below.

3.1. Study of slurry concentration

In direct sample introduction systems, the slurry concentration plays a very important role because it strongly affects the overall sensitivity. Very diluted slurries may cause degradation of the precision, while at high slurry concentrations the plasma stability and the nebulization efficiency of sample may be reduced significantly, due to the presence of bulk organic residues into the plasma. In preliminary experiments with direct introduction of 10% (m/v) slurry concentration, plasma was extinguished, independently of the values of the other parameters, such as sample flow rate, incident rf powder and nebulizer argon flow rate. This observation was confirmed using either the Cyclonic/Babington or the Scott/cross-flow configuration. In addition, slurry concentrations between 6 and 9% (m/v) produced severe clogging of the pump tubes and required frequent injector cleaning. The effect of slurry concentration was studied in the range 0.5–5.0% (m/v), using 1500 W RF incident power, 1.0 ml min^{-1} sample uptake flow rate, and 0.80 l min^{-1} nebulizer argon gas flow rate for the two nebulization configurations. Selected analytes like Ca (396.847 nm), Mg (280.271 nm) and Fe (238.204 nm), which are present in measurable amount in the examined samples, can be used for such study. The emission intensity of the above mentioned analytes is increasing linearly by increasing the slurry concentration.

Consequently, a compromise is needed for higher sensitivity, which is achieved when high slurry concentrations are used, and also for sufficient precision, which is favorable when low slurry concentrations are used. Thus, the slurry concentration was finally fixed to a moderate value of 2.5% (m/v) when using the Cyclonic/Babington configuration, and to 1.0% (m/v) when using the Scott/cross-flow configuration. Definitely, the first configuration is more efficient, because cyclonic spray chamber is more capable to transfer higher amounts of the sample solution and in addition the Babington type nebulizer performs better in high-solid solutions. The above two concentrations were selected respectively, for further optimization study in order to increase the overall sensitivity of the method with respect to the sample mass used.

3.2. Optimization of ICP parameters

RF power affects seriously the plasma temperature: the more RF power the hotter the plasma gets. Thus, RF incident power was studied for 1300, 1400 and 1500 W employing 1 ml min^{-1} sample uptake flow rate and 0.80 l min^{-1} nebulizer argon gas flow rate. As an example, the results for Ba and Cd are presented in Fig. 1. As it was proved, the highest sensitivity was obtained at 1500 W incident RF power for all analytes and both spectral lines, except for Al, Bi and Cr with the Cyclonic/Babington configuration and Bi, In, Ni with the Scott/cross-flow configuration, for which the sensitivities at 1400 and 1500 W were almost similar. According to the above observations, the incident power was fixed at 1500 W for further experiments.

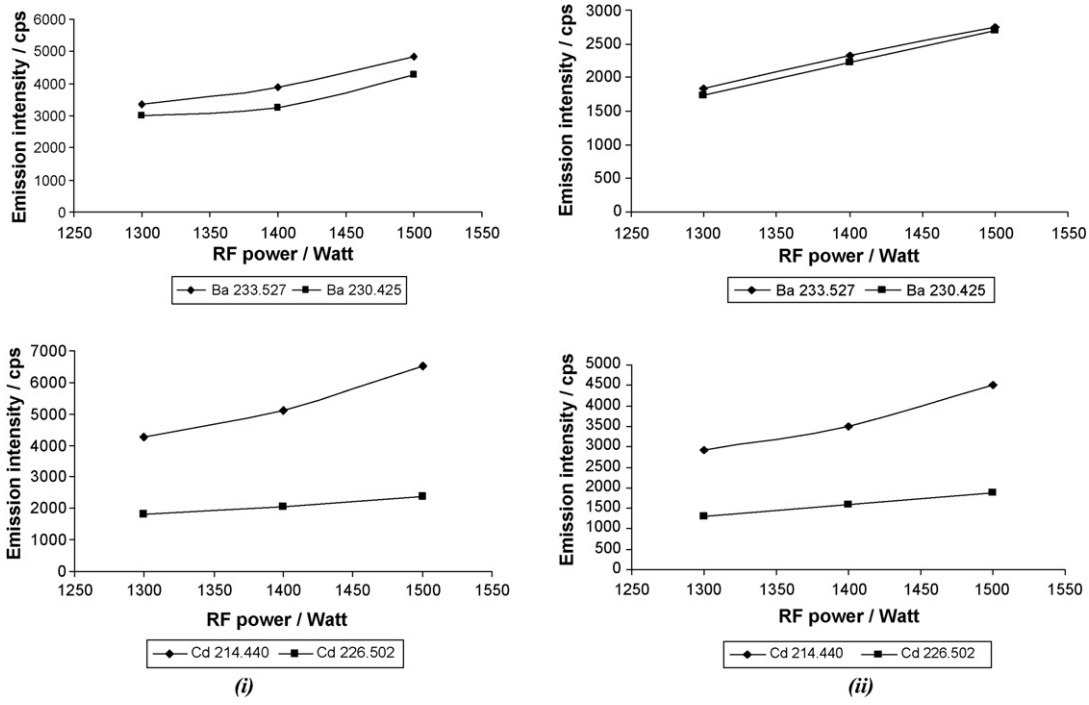

Fig. 1. Effect of the incident RF power on the emission intensity of Ba and Cd (200 μg l^{-1} each one), using (i) Cyclonic/Babington configuration (slurry concentration 2.5%, m/v) and (ii) Scott/cross-flow configuration (slurry concentration 1.0%, m/v).

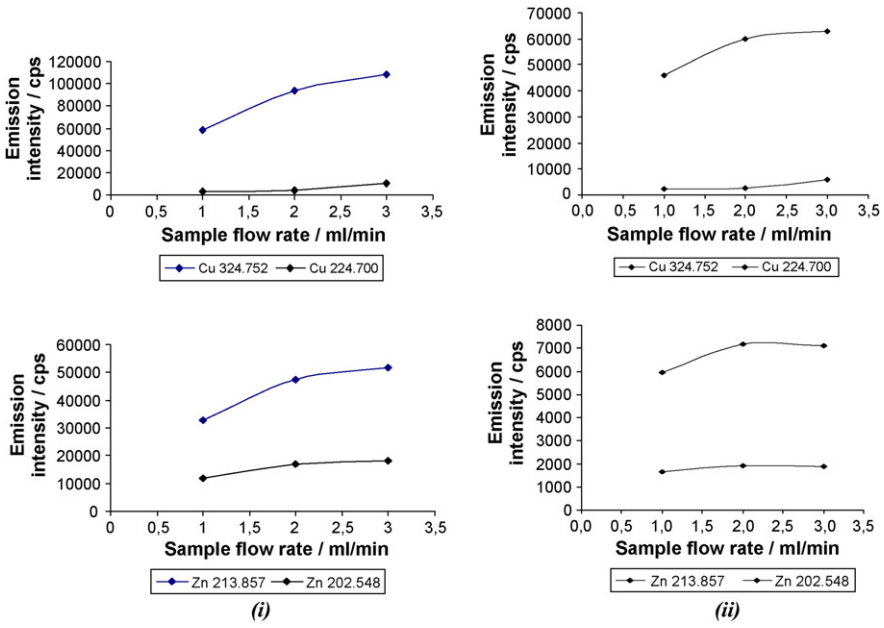

Fig. 2. Effect of the slurry sample flow rate on the emission intensity of Cu and Zn (200 μg l^{-1} each one), using (i) Cyclonic/Babington configuration (slurry concentration 2.5%, m/v) and (ii) Scott/cross-flow configuration (slurry concentration 1.0%, m/v).

Nebulizer gas flow rate and sample uptake flow rate affect seriously the slurry transportation into the ICP, the atomization and the excitation performance.

The effect of nebulizer gas flow rate was studied from 0.6 to 1.0 l min^{-1} appearing a maximum in the emission intensity at 0.8 l min^{-1} for both the examined nebulization configurations. Thus a 0.8 l min^{-1} nebulizer gas flow rate was adopted throughout.

The slurry sample flow rate was optimized in the range 1.0–3.0 ml min^{-1}. The results for, e.g. Cu and Zn, in two spectral lines, are given in Fig. 2. It was found that the signal intensity was increasing by increasing the sample flow rate for almost all

Table 3
Comparative regression data between aqueous calibration and standard addition curve of slurry aspiration for two spectral lines of each analyte and for two nebulization configurations

Analyte	Spectral line (nm)	Scott/cross-flow				Cyclonic/Babington			
		Aqueous standards calibration		Standard addition on slurry		Aqueous standards calibration		Standard addition on slurry	
		Slope	r	Slope	r	Slope	r	Slope	r
Ag	328.068	97.29	0.9994	107.9	0.9995	179.8	0.9995	207.1	0.9999
Al	308.215	12.83	0.9989	13.14	0.9941	47.17	0.9991	50.21	0.9920
As	193.696	0.388	0.9960	0.807	0.9547	1.00	0.9958	1.31	0.9865
Ba	233.527	14.11	0.9999	17.72	0.9982	36.54	0.9999	37.63	0.9995
Be	313.107	1090	0.9999	1220	0.9983	2256	0.9999	2130	0.9970
Bi	223.061	3.82	0.9988	4.02	0.9971	4.16	0.9991	4.51	0.9968
Ca	396.847	2634	0.9974	6161	0.9920	5482	0.9988	6404	0.9962
Cd	214.440	16.42	0.9999	21	0.9999	42.43	0.9999	37.81	0.9999
Co	228.616	9.01	0.9998	12.00	0.9967	18.70	0.9999	17.05	0.9945
Cr	283.563	47.51	0.9960	108.3	0.9971	123.0	0.9997	123.1	0.9957
Cu	324.752	166.8	0.9998	222.8	0.9999	301.8	0.9998	384.4	0.9994
Fe	238.204	14.87	0.9979	18.05	0.9992	67.40	0.9999	63.71	0.9992
Ga	294.364	11.36	0.9994	13.62	0.9999	18.91	0.9998	24.73	0.9995
In	325.609	6.99	0.9983	11.99	0.9945	15.04	0.9993	18.06	0.9952
Mg	280.271	421.4	0.9952	563.4	0.9945	1215	0.9994	1539	0.9960
Mn	257.610	170.9	0.9999	223.8	0.9997	420.1	0.9999	397.6	0.9995
Ni	232.003	2.91	0.9999	4.19	0.9980	6.41	0.9999	8.32	0.9976
Pb	220.353	0.471	0.9979	0.633	0.9942	1.14	0.9994	1.67	0.9869
Pd	340.458	18.56	0.9981	28.33	0.9959	42.07	0.9991	58.78	0.9999
Se	196.026	0.653	0.9991	1.06	0.9859	1.34	0.9979	1.88	0.9914
Zn	213.857	25.12	0.9998	33.21	0.9993	48.58	0.9995	57.49	0.9999

analytes. The behavior of all analytes was similar in both configurations tested. Thus for higher sensitivity, 3 ml min^{-1} was selected for the rest of the research.

3.3. Selection of nebulization configuration

The above defined optimum conditions (1500 W RF power, 3.0 ml min^{-1} sample uptake flow rate, 0.8 l min^{-1} nebulizer argon gas flow rate, slurry concentration 1.0 and 2.5% (m/v), for Cyclonic/Babington and Scott/cross-flow, respectively) were used in order to prepare calibration curves using aqueous multi-element standard solutions and standard addition calibration curves using spiked slurry solutions. Regression analysis was applied and the comparative results are given in Table 3.

The optimum spectral line for each analyte is presented in bold (Table 3). The most sensitive spectral line (higher slopes and $r > 0.99$) of each analyte was selected as optimum and used for further study and also for application of the method in various commercial antibiotic pharmaceuticals. In general, when aspirating slurries, the calculated correlation coefficients are slightly lower in comparison to those obtained using aqueous calibration standards, due to less reproducible analyte mass transportation to the plasma.

The slopes (S) of the obtained regression lines as they are listed in Table 3, were compared, and the sensitivity differences caused by the type of nebulization configuration for each analyte were calculated as the ratio $[100 \times (S_{cyclonic} - S_{scott})/S_{scott}]$ using: (a) aqueous standard solutions and (b) standard addition on slurries. It was proved that for all analytes there is a significant increase in the slope of both types of curves which varied in the range 0–350% when employing the Cyclonic/Babington configuration. Thus, the use of this type of nebulization configuration is preferred over the Scott/cross-flow one, and it is recommended in the proposed method. The latter can also be used but with poorer sensitivity for all analytes, usually two to three times lower. This differentiation arises because the cyclonic spray chamber combined with a Babington nebulizer allows higher rate of sample transportation into the plasma and is favorable when aspirating slurries with increased solid concentration.

3.4. Study of calibration technique

The slope differences caused by the type of calibration mode applied the ratio $[100 \times (S_{standadditon} - S_{aqueous})/S_{aqueous}]$, were calculated by comparing the slopes obtained when employing: (a) Cyclonic/Babington and (b) Scott/cross-flow configurations, respectively, as they are listed in Table 3. It was proved that for the majority of the analytes there is an increase in the slope between 0 and 50% when the Cyclonic/Babington configuration is used and between 0 and 150% when the Scott/cross-flow configuration is employed. These observations proved that the Cyclonic/Babington configuration could compensate more efficiently the effect of the slurry matrix. However, the increase in the sensitivity was in contrast to what was initially expected, i.e. a negative effect was expected for all analytes, due to the aspiration of slurry into the plasma, but this was true only for some analytes, for which a slight negative effect was observed.

Table 4
Adsorption (%) of several elements from solutions containing 10.0 mg l^{-1} of each element and 1% (m/v) suspended antibiotic powder as slurry in 0.5 mol l^{-1} HNO_3

Type of antibiotic	% adsorption					
	Fe	Mn	Cu	Cr	Co	Ni
AMC	17.3	18.6	18.5	42.8	13.8	8.8
CTM	19.1	11.1	9.1	15.0	17.4	11.5

AMC: sample containing amoxicillin and excipients; CTM: sample containing clarithromycin and excipients.

The slope's increase is explained because, during slurry formation by using a mixed-element standard solution, the suspended sample particles adsorb analytes or in some cases forms metal-complexes, leading thus to preconcentration of the analytes in the slurry. This fact was confirmed by independent investigation of the adsorption and/or complexation capacity of two antibiotic drugs containing amoxicillin and clarithromycin as APIs. Fe, Mn, Cu, Cr, Co and Ni where determined by flame atomic absorption spectrometry in the supernatant obtained after centrifugation of 1% (m/v) slurries containing 10 mg l^{-1} of each element. It was proved that after 1 min stirring, the supernatant standard solution has lost 9.1–42.8% of the original concentration of each analyte (Table 4). This behavior is variable and depends on the chemical form of the API of antibiotics, which is capable to form stable metal complexes. For this reason, the use of aqueous standards calibration is considered more precise and reproducible than the standard addition spiked slurries, and it is recommended.

3.5. Robustness test

A quantitative method for multi-element analysis of antibiotics must be applicable to various types of samples. Commercial antibiotics contain diverse types of APIs (penicillins, cephalosporins, etc.) and also numerous excipients. The performance of the method should not be sensitive to all these ingredients. For this reason the proposed slurry aspiration method was tested for its robustness against four different antibiotic formulations. The variation of the slope of the standard addition curves obtained is presented in Fig. 3. The overall stability of the level of the slope of almost all the analytes proved that the nebulization of slurries is efficient and reproducible. Minor variations (less than 10% for the majority of analytes) may be due to the different extent of metal complexation or absorption either by the APIs or by the included excipients, as it was described above.

3.6. Detection limits, accuracy and precision

Under the above described optimum and recommended conditions, the detection limits (LOD, $\mu g\,g^{-1}$) were calculated using the 3 s criterion (three times the standard deviation of 10 blank measurements), while the quantitation limits (LOQ, $\mu g\,g^{-1}$) were calculated using the 10 s definition, taking into account 2.50% (m/v) sample concentration in the slurry and the

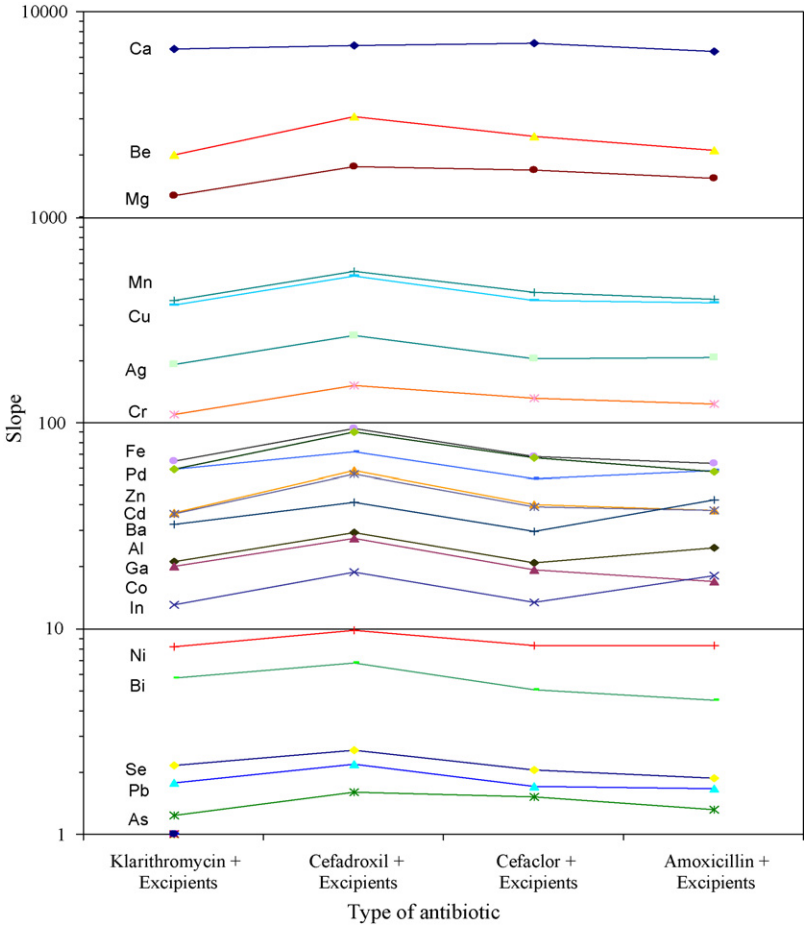

Fig. 3. Robustness test for the slope, when the proposed method is applied to several commercial antibiotics with various excipients (the connecting lines are drawn just to avoid confusing the behavior of each analyte).

use of Cyclonic/Babington configuration. The detection limits for the most sensitive spectral line of each analyte are presented in Table 5. The quantitation limits (LOQ) are nearly three-fold the corresponding detection limit for each analyte. It can be seen that for all analytes the capability of the method is satisfactory, and can be applied either as a quantitative method or as a screening one.

The precision, expressed by means of relative standard deviation of 10 replicates at 100 $\mu g\,l^{-1}$ concentration level, was also calculated and was ranged between 3.3 and 8.1% for almost all analytes. Due to the fact that no sample pretreatment is required, a sample measurement frequency of 20 h^{-1} is easily achieved.

The accuracy of the proposed method was evaluated versus complete wet-acid digestion of commercial antibiotic containing amoxicillin as API and a variety of excipients, because no reference material with a multi-element certification is commercially available. The results of the wet digestion method were assumed as reference in order to estimate the error of the slurry introduction method. The comparative results concerning analytes which can be quantitatively determined are given in Table 5. All other analytes were below their detection limit by both methods.

Table 5
Comparative results (mean concentration ± standard deviation) of the slurry aspiration method vs. complete acid digestion method for sample containing amoxicillin and excipients

Analyte[a]	LOD ($\mu g\,g^{-1}$)	Slurry technique (μg/g)	Wet-acid digestion (μg/g)	Error (%)
Al	0.48	(1.1)[b]	(1.0)	–
Ca	0.04	8.2 ± 0.6	7.7 ± 0.2	+6.5
Cu	0.20	(0.4)	(0.4)	–
Fe	0.16	1.5 ± 0.1	1.4 ± 0.1	+7.1
Mg	0.08	94 ± 5	89 ± 6	+5.3
Mn	0.16	(0.30)	(0.34)	–
Zn	0.23	0.75 ± 0.10	0.81 ± 0.06	−7.4

[a] All other analytes were below the detection limits.
[b] Values in parentheses are given as estimation only, because they are higher than limit of detection but lower than limit of quantitation.

Table 6
Analysis of commercial antibiotics by ICP-AES and the slurry technique ($\mu g\,g^{-1}$)

Analyte	LOD ($\mu g\,g^{-1}$)	CFC ($\mu g\,g^{-1}$)	CFD ($\mu g\,g^{-1}$)	CTM ($\mu g\,g^{-1}$)
Ag	0.11	<LOD	<LOD	<LOD
Al	0.48	<LOD	(0.6)	<LOD
As	1.92	<LOD	<LOD	<LOD
Ba	0.37	<LOD	<LOD	<LOD
Be	0.08	<LOD	<LOD	<LOD
Bi	0.79	<LOD	<LOD	<LOD
Ca	0.04	44 ± 4	59 ± 5	25 ± 3
Cd	0.16	<LOD	<LOD	<LOD
Co	0.40	<LOD	<LOD	<LOD
Cr	0.17	<LOD	<LOD	<LOD
Cu	0.20	(0.3)[a]	<LOD	0.86 ± 0.12
Fe	0.16	1.7 ± 0.1	1.0 ± 0.1	2.2 ± 0.3
Ga	0.80	<LOD	<LOD	<LOD
In	0.60	<LOD	<LOD	<LOD
Mg	0.08	424 ± 20	78 ± 16	6 ± 2
Mn	0.16	(0.3)	<LOD	<LOD
Ni	0.60	<LOD	<LOD	<LOD
Pb	2.39	<LOD	<LOD	<LOD
Pd	0.25	<LOD	<LOD	<LOD
Se	2.08	<LOD	<LOD	<LOD
Zn	0.23	1.2 ± 0.1	0.8 ± 0.1	1.5 ± 0.2

[a] Values in parentheses indicate detectable levels, but not quantitatively determined.

3.7. Application to commercial antibiotic formulations

The developed method was applied to the determination of trace elements in commercial antibiotics, containing cefaclor (CFC), cefadroxil (CFD) and klarithromycin (CTM) as APIs and various other excipients like dimethicone, magnesium stearate, starch maize, gelatin, titanium dioxide, lactose, flavors, citric acid, etc. The results are presented in Table 6. It is reminded that Table 5 lists also the results of concerning another type of antibiotic formulation, with amoxicillin as API.

It was shown that the examined antibiotics did not contained detectable concentrations of toxic heavy metals like Cd, Pb, Ag, Pd, etc. Among the other investigated analytes, only Ca, Mg, Fe and Zn were found in measurable levels in all formulations, while Al was detected only in sample CFD, Mn in CFC and Cu in CFC and CTM samples.

4. Conclusions

The direct nebulization technique of slurry sample into the plasma nebulizer was proved an efficient method for analysis of commercial antibiotic drugs by ICP-AES, independently of their other matrix constituents. The developed method can be used for quantitative determination of 21 analytes down to the $\mu g\,g^{-1}$ level. Slurry solutions in the range up to 2.5% (m/v) can be easily aspirated without significant effect on plasma and baseline stability, using the cyclonic spray chamber combined with a Babington-type nebulizer. Combination of a scott-spray chamber and a cross-flow nebulizer was less tolerable to slurry aspiration. In the latter case slurries containing up to 1.0% (m/v) powder could be sufficiently aspirated. By direct introduction of high mass slurries it is possible to achieve sufficient sensitivity, comparable to those of other more expensive multi-element techniques. The method of standard addition is not to be used because adsorption and preconcentration of analytes by the active ingredients of the suspended matter. No sample pretreatment is needed, thus the method is convenient for routine and quantitative analysis of antibiotics in powder forms.

References

[1] G. Mukherjee, T. Ghosh, J. Inorg. Biochem. 59 (1995) 827–833.
[2] N. Nikolis, C. Methenitis, G. Pneumatikakis, M.M.L. Fiallo, J. Inorg. Biochem. 89 (2002) 131–141.
[3] L.-J. Ming, J.D. Epperson, J. Inorg. Biochem. 91 (2002) 46–58.
[4] P. Gutierez Navarro, A. El Bekkouri, E. Rodriguez Reinoso, Analyst 123 (1998) 2263–2266.
[5] N.R. Chatterjee, M.S. Degani, C.B. Singh, Indian J. Pharmaceut. Sci. 50 (1988) 128–130.
[6] European Agency for Evaluation of Medicinal Products, Evaluation of medicines for Human Use, Note for guidance on specification limits for residues of metal catalysts, London, 2002.
[7] European Pharmacopoeia, 5.2 Analytical Methods, Date of Implementation 2005, pp. 103–105.
[8] British Pharmacopoeia, Appendix, Norwich, UK, 2000, pp. A174–176.
[9] Japanese Pharmacopoeia, Method 22, XIV ed., Part I, pp. 43–44.
[10] J.F. van Staden, C.J. Hattingh, Fresen. J. Anal. Chem. 367 (2000) 79–83.
[11] M. Niemela, H. Kola, K. Eilola, P. Peramaki, J. Pharm. Biomed. Anal. 35 (2004) 433–439.
[12] A.I. Stoica, M. Peltea, G.-E. Baiulescu, M. Ionica, J. Pharm. Biomed. Anal. 36 (2004) 653–656.
[13] A. Kelko-Levai, I. Varga, K. Zih-Perenyi, A. Lasztity, Spectrochim. Acta B 54 (1999) 827–833.
[14] L. Wang, M. Marley, H. Jahansouz, C. Bahnck, J. Pharm. Biomed. Anal. 33 (2003) 955–961.
[15] T. Wang, Z. Ge, J. Wu, B. Li, A. Liang, J. Pharm. Biomed. Anal. 19 (1999) 937–943.
[16] B. Budic, S. Klemenc, Spectrochim. Acta B 55 (2000) 681–688.
[17] G. Zachariadis, D. Kapsimali, J. Pharm. Biomed. Anal. 41 (2006) 1212–1219.
[18] N. Lewen, S. Mathew, M. Schenkenberger, T. Raglione, J. Pharm. Biomed. Anal. 35 (2004) 739–752.
[19] T. Wang, J. Wu, R. Hartman, X. Jia, R.S. Egan, J. Pharm. Biomed. Anal. 23 (2000) 867–890.
[20] A. Lasztity, A. Kelko-Levai, I. Varga, K. Zih-Perenyi, E. Bertalan, Microchem. J. 73 (2002) 59–63.
[21] E.G. Yanes, N.J. Miller-Ihli, Spectrochim. Acta B 60 (2005) 555–561.