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A rapid ICP-MS screen for heavy metals in pharmaceutical compounds

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Abstract

A robust general inductively coupled plasma-mass spectrometry (ICP-MS) based method was developed as an alternative to the wet chemical heavy metals test prescribed in the United States Pharmacopoeia (USP), British Pharmacopoeia (BP), Japanese Pharmacopoeia (JP) and European Pharmacopoeia (EP). The described method provides specific detection and quantitation for each of the elements expected to give rise to a positive response in the compendial methods: arsenic (As), selenium (Se), cadmium (Cd), indium (In), tin (Sn), antimony (Sb), lead (Pb), bismuth (Bi), silver (Ag), palladium (Pd), platinum (Pt), mercury (Hg), molybdenum (Mo) and ruthenium (Ru). The subjectiveness of the visual based semi-quantitative comparison that is performed in the compendial methods is eliminated through the utilization of the ICP-MS. The described method has been in use for several years and its versatility has been demonstrated by successfully applying it to a wide variety of sample matrices. Analysis of the specific elemental data from the numerous sample matrices investigated indicates that there is no dependence of the various chemical functionalities contained in the sample matrices on the individual element recoveries. The average recovery for each element from the various sample matrices investigated ranged from 89 to 102%.

Keywords: Heavy metals; Compendial methods; Inductively coupled plasma-mass spectrometry (ICP-MS); Active pharmaceutical ingredient (API); Analysis; General method

1. Introduction

As the pressures to accelerate the pharmaceutical development process have increased, analysts and chemists have been pressured to develop chemical processes, process controls and appropriate test methods on shorter and shorter time lines. One staple that has remained in the characterization and specifications of active pharmaceutical ingredients (APIs) has been the inclusion of an end of processing control to determine potential metal contamination using one of the compendial heavy metals test. These tests have been included, historically, to ensure that no inorganic-based non-process related contaminants were introduced into the sample at any of the numerous processing steps used to produce the material. The United States Pharmacopoeia (USP), British Pharmacopoeia (BP), Japanese Pharmacopoeia (JP) and European Pharma-

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copoeia (EP) compendial methods [1–4] have been used for many years to collectively monitor the total arsenic (As), selenium (Se), cadmium (Cd), indium (In), tin (Sn), antimony (Sb), lead (Pb), bismuth (Bi), silver (Ag), palladium (Pd), platinum (Pt), mercury (Hg), molybdenum (Mo) and ruthenium (Ru) content in pharmaceutical materials. While Pd, Pt, Se and Ru are not elements listed in the various pharmacopoeia, they are included in this listing because they might be expected to respond to the compendial tests.

The current compendial methods were developed before the advent of modern analytical instrumentation and are based on wet chemical techniques, which can be easily transferred from one laboratory to another and do not require expensive instrumentation or highly trained laboratory personnel to perform them. However, because the methods rely on a subjective visual examination and comparison of the sample solutions to a lead standard solution, they require large amounts of sample to obtain parts per million ($\mu g\,g^{-1}$) detection levels. Additionally, the methods provide no qualitative or element-specific information, and usually involve a heating or ashing step, which is known to cause losses of the volatile elements. After ashing the sample, the compendial methods utilize a reaction to form the sulfide species of any elements which may be present. The resulting solution is then visually compared to a lead standard solution that has been treated identically. The total heavy metal content is reported versus the lead standard response as a limit test, e.g. $<20\,\mu g\,g^{-1}$. Because of the similarities between the USP, EP, JP and BP methods, for the purposes of this paper, all subsequent references to compendial methods refer to the USP method.

The compendial method is based on the assumption that each of the 14 elements present in the sample matrix will react with thioacetamide 100% or to the exact extent as the lead standard to form a sulfide species. The insolubility of most sulfides has long been used in remediation efforts in the environmental field, where heavy metals are precipitated to remove them from soils, waters and other contaminated areas [5–8]. In the same way, the compendial method assumes that any sulfides generated in the sample will form a precipitate which can then be compared to the precipitate formed by the lead standard. The compendial method also assumes that the reaction kinetics for the formation of the potential sulfides are very similar to the reaction kinetics for the formation of lead sulfide in the standard solution and that the reaction kinetics are not impacted significantly by the sample matrix. Since many metals sulfides can form colloids, the compendial method requires that the visual sample comparison be performed in a relatively short amount of time after the precipitate has formed ($\sim$5 min) to minimize any effects that Ostwald ripening [9] may cause. Lastly, since the compendial method relies on a visual comparison, it assumes that the visual characteristics of the potential sulfides formed are similar enough to the lead sulfide and unaffected by the sample matrix to be considered essentially identical.

One crucial assumption that is not mentioned above is that the heating and/or ashing step that the sample must undergo does not result in the loss of any of the elements of interest and precludes them from forming precipitates or colloids for comparison. For the volatile elements, such as mercury and selenium, this assumption lacks scientific merit, and given the known toxicological effects of these elements, highlights the need for the development of a more reliable and accurate method. A USP committee on the "Harmonization of the USP, EP and JP Heavy Metals Testing Procedures" acknowledged several of the shortcomings in the compendial methods in its statement:

> It was concluded from this experiment that approximately 50% of the metals may be lost during the ash process. The loss of metals is probably matrix-dependent, and because the procedures are very labor-intensive, recoveries could vary significantly among analysts. Note that mercury, which is one of the more toxic heavy metals, was not recovered from either set of samples.
>
> Because of the loss of metals during ignition, the validity of the test results obtained with the current USP, JP and EP general test procedures is questionable [10].

Difficulty in obtaining reliable and reproducible results from the compendial method is not limited to the heating and sulfide formation steps. In addition, analysts can differ in their determination of a result by how they perform the visual comparison. Inexperienced analysts may not understand the subtleties of how to accurately and consistently read the sample and standard solutions each time, including the im-

pact of the length of time that the sample is allowed to sit prior to reading, the extent to which the samples have undergone colloid formation, the subtlety in the visual difference between the different lead standards and the effect that the laboratory lighting can have on the interpretation of the visual results. As mentioned previously, the comparison to a single element standard precludes the ability to provide element-specific information. Consequently, results obtained from the compendial method are provided as a limit test, and do not give the analyst any indication as to which elements are the cause for an higher than expected result. Thus, the compendial method provides the analyst with limited assistance in tracing the result back to its potential source. Lastly, since the compendial method requires the use of large amounts of sample (∼1 g), its application at the early stages of drug development is difficult, due to the very limited supply of material.

Recognizing the issues associated with the compendial method that have been outlined above, an investigation was initiated within our laboratories to develop a spectroscopic alternative to the compendial method [11]. For regulatory reasons the spectroscopic method was developed to mimic the compendial method as closely as possible so that data generated at the early stages of drug development using the spectroscopic method could be compared directly to data generated by the compendial methods. Additionally, it was desired that the new method provide the analyst with element-specific quantitation of the sample and the ability to automate the method.

Inductively coupled plasma-mass spectrometry (ICP-MS) was selected as the basis of the alternative method since it provides good sensitivity, requires minimal sample size, affords minimal elemental interferences and readily provides a means to perform rapid and automated multi-elemental analyses. Because ICP-MS provides the analyst with a wide range of element selectivity, the analyst may opt to include as many elements as possible when developing a given method, a strategy applied by Wang et al. in their paper [12]. However, since the goal in this investigation was to develop a spectroscopic alternative to the compendial method the inclusion of elements not associated with the compendial methods would unnecessarily bias the results and preclude a direct comparison.

Detection limits (DL) in the ppb and sub-ppb levels are commonly achieved with ICP-MS for many of the elements of interest. This sensitivity allows the analyst to utilize less concentrated sample solutions, thereby eliminating the need for large sample sizes and minimizing the potential effects that the sample matrix may have on the result. In developing a method that is intended to be used with samples consisting of a wide variety of organic molecules including salts, free acids and bases, it is highly desirable to minimize the sample matrix effects as much as possible by sample dilution. The alternative ICP-MS method described in this paper takes advantage of this sensitivity by utilizing 40 times less sample than the compendial method (0.025 g versus 1 g) and using solutions containing only 1 mg ml^{-1} of the sample matrix.

2. Experimental

2.1. Reagents and materials

Concentrated nitric (Ultrex II grade) and hydrochloric (Ultrex II grade) acids were purchased from VWR Scientific (West Chester, PA 19380). Dimethylsulfoxide (DMSO, SpectrAR grade) and 2-butoxyethanol (OR grade) were purchased from Mallinckrodt (St. Louis, MO 63134). Type I water (18 mΩ) was prepared by passing deionized water through a Milli-Q water system (Millipore, Billerica, MA). Certified, NIST-traceable ICP stock standard solutions containing the elements of interest: Ag, As, Se, Sn, Sb, Pd, Cd, In, Pt, Pb, Bi, Hg, Ru and Mo at concentrations of 1000 or 100 μg ml^{-1} were purchased from Inorganic Ventures Inc. (Lakewood, NJ 08701).

The reagents used to prepare the samples and standards for the USP compendial method: thioacetamide-glycerin base TS, lead nitrate, ammonium acetate, hydrochloric acid, ammonium hydroxide, sulfuric acid, nitric acid and acetic acid, were all purchased from VWR Scientific (West Chester PA, 19380) and were reagent grade.

Sample matrices referenced in this paper refer to new investigational drug substances or intermediates under development, and were obtained from the Bristol-Myers Squibb Process Research and Development laboratories (Bristol-Myers Squibb, New Brunswick, NJ 08903).

2.2. Solvent selection

ICP-MS is a technique that performs best when samples are dissolved in deionized water or dilute nitric acid. In developing a general method, it was necessary to provide a solvent system suitable for use with a wide range of organic sample matrices that would be encountered, regardless of the functional groups that may be contained in the sample matrix. Since many of the APIs received by our laboratory are not sufficiently soluble in deionized water or dilute nitric acid, several different solvents were studied. The solvents investigated were water, 2-butoxyethanol:water (25:75), 5% nitric acid, 2% nitric acid, 75% DMSO, 0.5% hydrochloric acid, and hydrochloric acid:2-butoxyethanol:water (2:25:73).

For each solvent investigated, a series of standards, ranging from 0 to 25 ng ml^{-1} (equivalent to 25 μg g^{-1} in the solid) was assayed to demonstrate linearity. Of the seven solvents originally studied, three (75% DMSO, 2% nitric acid and hydrochloric acid:2-butoxyethanol:water (2:25:73)), provided <90% recovery for the elements in the method, which was considered unacceptable. These solvents were removed from consideration as possible dissolution solvents for the method. Fig. 1 shows the average recoveries for the remaining four solvents: deionized water, 2-butoxyethanol:water (25:75), 0.5% hydrochloric acid and 5% nitric acid. While overall standard recoveries are better for 0.5% hydrochloric acid than the remaining three solvents, many of the APIs being studied are not soluble in 0.5% hydrochloric acid. For this reason, work focused on the solvent which provided the next best average standard recoveries for each element, 2-butoxyethanol:water (25:75).

2.3. Preparation of standard solutions

Working standard solutions were prepared containing 10 and 20 ng ml^{-1} each of the elements of interest by diluting the individual stock standard solutions, excluding Ag, in 2-butoxyethanol:water (25:75). A separate set of working standard solutions was prepared containing only Ag, since this metal was observed to precipitate with the nitrate counterion associated with several of the other elements in the standard solutions. The tuning solution for the ICP-MS instruments contained 25 ng In ml^{-1}. All standard and sample solutions contained 25 ng ml^{-1} each of cobalt (Co), gold (Au) and rhodium (Rh) as internal standards.

2.4. Preparation of sample solutions for the USP Heavy Metals test

Working sample solutions for the USP Heavy Metals test were prepared by accurately weighing approx-

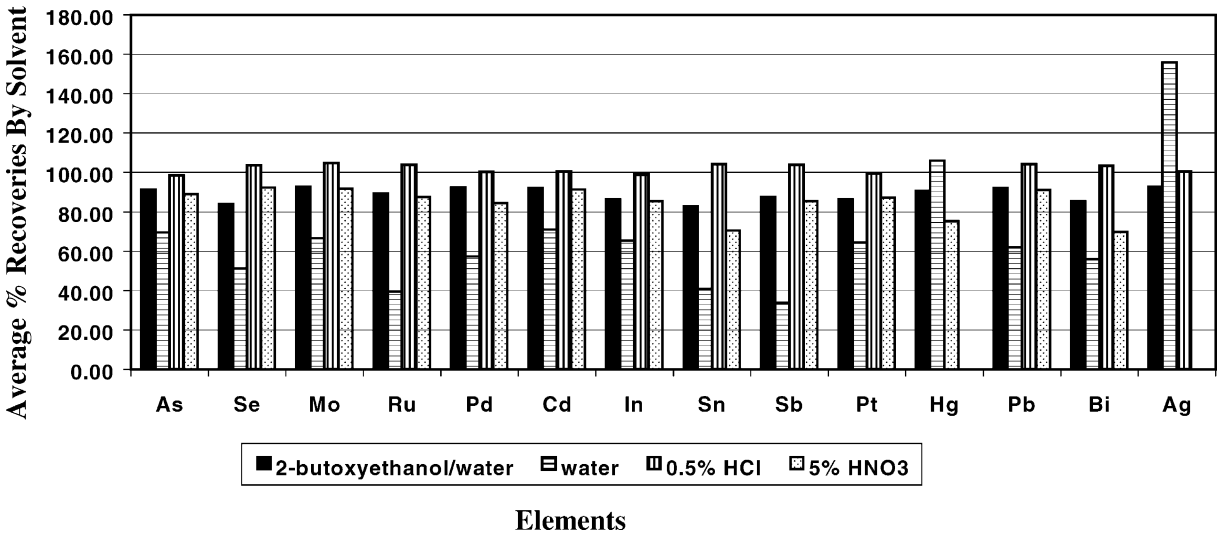

Fig. 1. Comparison of the average (%) recoveries of the individual elements in different solvents (by ICP-MS).

imately 1 g of sample into a crucible and following the sample preparation outlined in the USP Heavy Metals, II procedure. Spiked samples were prepared to contain 10 $\mu g\,g^{-1}$ of each element by directly spiking the solid sample with an appropriate volume of the stock standard solutions prior to initiating the sample preparation step for the USP procedure. The sample solutions were compared to an appropriate lead standard solution prepared as per the USP Heavy Metals, II procedure and the result recorded.

2.5. Preparation of sample solutions for ICP-MS analysis

Working sample solutions containing 1 mg ml^{-1} of the sample matrix investigated were prepared by accurately weighing approximately 25 mg of the sample into a 25 ml volumetric flask, adding the equivalent of 25 ng ml^{-1} each of cobalt (Co), gold (Au) and rhodium (Rh) as internal standards and diluting with 2-butoxyethanol:water solution (25:75). Spiked sample solutions were prepared at the concentration of 10 $\mu g\,g^{-1}$ of each analyte element.

Each of the final sample solutions from the USP Heavy Metals test was diluted 1:5 with a solution of 2-butoxyethanol:water (25:75), and contained 25 ng ml^{-1} each of cobalt (Co), gold (Au) and rhodium (Rh) as internal standards and assayed by ICP-MS.

2.6. Instrumentation

Both a VG Elemental PlasmaQuad Turbo II+ ICP-MS and a Micromass Platform ICP-MS were used in this study. Default instrument parameters were selected for both instruments. Table 1 lists the specific operating parameters used for both instruments. To monitor for potential spectral interferences, multiple isotopes were used for those elements that were not monoisotopic. Table 2 lists the isotopes that were monitored for each element. The ICP-MS systems were tuned using a solution containing 25 ng In ml^{-1}. The instruments were calibrated using working standard solutions that contained 10 and 20 ng ml^{-1} of each element investigated.

Table 1
Instrumental parameters used

Parameter	VG PlasmaQuad PQII Turbo Plus ICP-MS	Micromass Platform ICP-MS
Sampling cone	Platinum	Platinum
Starting mass	59 amu	59 amu
Ending mass	209 amu	209 amu
Number of channels	20	32
Collector type	Pulse counting	N/A
Measurement mode	Peak jumping	Peak jumping
Dwell (s)	0.20	0.224

Table 2
Elemental isotopes monitored

Analyte element	Isotope
As	75
Se	76, 80, 82
Mo	95
Ru	99, 102
Pd	106, 108
Cd	111, 114
In	115
Sn	118, 120
Sb	121
Pt	194, 195
Hg	200, 202
Pb	206, 207, 208
Bi	209
Ag	107, 109
Co (internal standard)	59
Au (internal standard)	197
Rh (internal standard)	103

2.7. Experimental procedure

Following the tuning and calibration of the ICP-MS system being used, the working sample solutions were assayed for As, Se, Sn, Sb, Pd, Cd, In, Pt, Pb, Bi, Hg, Ru and Mo. Standard solutions were assayed after every fifth sample, and their concentrations had to agree to within ±20% of their theoretical value or the instrument was recalibrated. Following the analysis of the first 13 elements, the instrument was recalibrated with the silver standards for the analysis of silver, with the same acceptance criteria for that element as for the previous 13 elements. The results of multiple isotopes were averaged, and the results obtained for each element were added together to obtain a total heavy metals concentration. If a given isotope provided drastically different results than the others, this result was further investigated.

In addition to the analysis of the unspiked sample solutions, several samples ($n = 9$) were also spiked to a concentration of 10 ng ml^{-1} (equivalent to 10 μg g^{-1} in the solid) or 20 ng ml^{-1} (equivalent to 20 μg g^{-1} in the solid) of each of the analyte elements. In the case of silver, a separate spiked sample was prepared to avoid precipitation of the silver from the sample solution. Spike recoveries had to agree with their theoretical values to within ±50%.

For purposes of comparison, samples were also assayed using the USP Heavy Metals test, II ($n = 9$). These samples were also spiked to a concentration of 10 ng ml^{-1} (equivalent to 10 μg g^{-1} in the solid) for each of the analyte elements, for a total spike concentration of 140 ng ml^{-1} (equivalent to 140 μg g^{-1} in the solid). Upon the completion of these analyses, the resultant sample solutions were retained for analysis by ICP-MS, to ascertain whether the USP test is capable of detecting the elements being monitored by the ICP-MS heavy metals screen.

The calculated detection limits and minimum quantifiable limits (QL) for each of the analytes in 2-butoxyethanol:water (25:75) are shown in Table 3. It is important to note that the absolute determination of QL and DL values may not be appropriate for such a general method, where it would be more useful to perform a single QL spiked analysis when analyzing an API for the first time [13]. For each new API analyzed by the ICP-MS heavy metals screen, a single sample was spiked to a level of 10 μg g^{-1} (equivalent to 10 μg g^{-1} in the solid), to ensure adequate sensitivity for each element. The instrument is capable of providing more sensitive data, but in keeping with the desire to mimic the compendial methods as much as possible, it was determined that lower QLs were not required.

Table 3
DL and QL values in 2-butoxyethanol:water (25:75)

Element	Calculated DL, $n = 10$ (μg g^{-1})	Calculated QL, $n = 10$ (μg g^{-1})
As	0.37	0.94
Se	0.42	1.41
Mo	0.08	0.08
Ru	0.20	0.68
Pd	0.18	0.60
Cd	0.03	0.10
In	0.17	0.57
Sn	0.35	1.17
Sb	0.16	0.54
Pt	0.03	0.10
Hg	1.82	6.07
Bi	1.51	5.03
Ag	0.15	0.50

3. Results and discussion

3.1. USP Heavy Metals procedure, II versus ICP-MS procedure

It is well known that the USP Heavy Metals test is plagued by the inadequate recoveries of the elements of interest. Recent papers [10,12] have referenced this issue as well. However, there has been limited documentation of the poor recovery and inappropriateness of the test in the literature to date. Early in the development of the described method, several different samples were prepared to evaluate the appropriateness of the USP and the alternative ICP-MS methods. Nine different drug substance sample matrices were prepared and analyzed according to the USP Heavy Metals procedure. In addition, each sample was divided before preparation and spiked with the equivalent of 10 μg g^{-1} of each of the 14 elements that are monitored in the USP method and then analyzed using the compendial method. Two of the sample matrix blanks generated cloudy solutions that could not be read using the USP method. All of the other sample matrix blanks were determined by the USP method to have a total heavy metals content of <10 μg g^{-1}. Despite the fact that all of the spiked sample solutions contained a total of 140 μg g^{-1} of the elements investigated (10 μg g^{-1} of each element), none of the spiked sample solutions was determined by the USP test to have a total heavy metals content of more than 80 μg g^{-1}. One of the spiked sample matrices was determined to have a total heavy metals content of >60 and <80 μg g^{-1}. All of the other spiked sample matrices were determined to have a total heavy metals content of >40 and <60 μg g^{-1}. These results were not surprising due to the extreme conditions that the sample is subjected to during the USP test.

To confirm the actual total heavy metals content of the blank and spiked sample solutions used to evaluate the USP Heavy Metals test and to determine which specific metals, if any, are lost during the sample work

up, each of the resulting blank and spiked sample solutions was analyzed by ICP-MS. Prior to ICP-MS analysis, internal standard was added to each solution and the solution was diluted with 2-butoxyethanol:water (25:75). Analysis of these solutions would show if the elements assumed to give rise to a response in the USP method were actually present in the samples but were just undetected (or underdetected) by the USP testing procedure.

A close inspection of the results from all nine sample matrices indicated that the average recoveries for several of the elements including selenium, tin, antimony, ruthenium and mercury were all significantly less than 10%. Equally alarming were the recoveries for the elements lead, arsenic, cadmium, molybdenum, palladium, platinum and indium whose averages all fell between 30 and 50%. Silver was the only element whose recovery from the spiked sample solutions was respectable, averaging 97%. It is interesting to note that the USP Heavy Metals, II procedure calls for the sample solutions to be compared to a similarly treated lead standard solution. In the spiked sample solutions, the average recovery of lead was ∼50%, indicating that a comparison to a lead standard may not provide optimal results for any given sample.

The poor recovery of the more volatile elements is not unexpected, given the high temperatures that the sample is subjected to during the USP method (∼500–600 °C). Eight of the elements have melting points of ∼600 °C or less. Clearly, subjecting the sample matrix to extreme temperatures would not be expected to provide accurate and consistent results for the more volatile elements and poor recoveries of these elements is to be expected.

To determine the recovery efficiency of the alternative ICP-MS heavy metals screen method described, blank sample and spiked sample solutions were prepared for each of the same sample matrices investigated with the USP method. The spiked sample solutions were prepared by adding 10 $\mu g\,g^{-1}$ of each of the 14 elements monitored in the USP method for a total heavy metals concentration of 140 $\mu g\,g^{-1}$. Excellent recoveries were obtained for each of the 14 elements monitored. Fig. 2 provides a graphical comparison between the average recoveries obtained for the different spiked sample solutions prepared using the USP Heavy Metals, II testing procedure and those prepared using the ICP-MS procedure.

3.2. Generality of the method

To assess the generality of the described ICP-MS alternative test, the individual element recovery data collected from the different sample matrices investi-

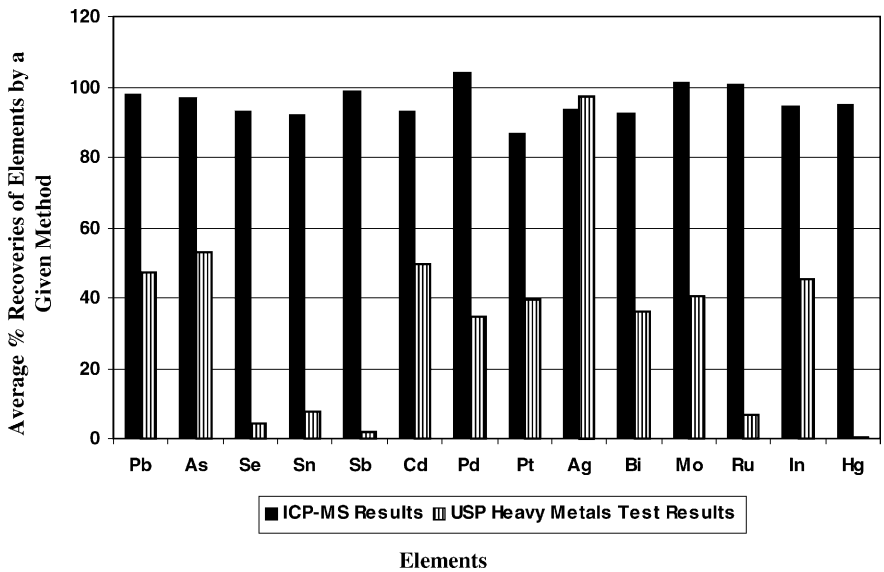

Fig. 2. Comparison of average (%) recoveries of elements: USP Heavy Metals test vs. ICP-MS Heavy Metals test.

gated was examined in more detail. Since the compounds investigated were all new investigational drug substances their chemical structures are proprietary and cannot be disclosed. Therefore, the following system was devised for documenting the differences in the chemical functionalities contained in each of the sample matrices. First, each sample matrix was assigned an unique integer starting with the number one. Then a value of one was assigned for each time a particular functional group was present in a molecule. For example, if a molecule contained two primary amine groups (RNH_2) the structure was assigned a value of two for primary amines. Table 4 provides a complete list of the chemical functionality data for the different sample matrices investigated along with the individual element recovery data for each sample matrix. If a particular chemical functionality occurred in only one sample matrix that chemical functionality was not listed in the table.

To assess what impact a particular functional group may have had on the recovery of an element, the average recovery for each element in the matrices that contained the particular functional group being examined was compared to the average recovery of each element determined from all of the sample matrices investigated. The differences observed for each element by functional group were then determined for that particular chemical functionality and plotted as shown in Fig. 3. All element recoveries for these groupings were within ±23% of the overall recoveries. An inspection of the plot in Fig. 3 indicates that, in general, the average individual element recoveries for each of the functional groups investigated are within ±10% of the average recoveries from all of the sample matrices investigated, with the following exceptions: the recovery of several of the elements in the matrices that contain the functional groups RSH, RSR, F, COOH and RCOOR. A close inspection of the data indicates that relatively few matrices were evaluated that contained these functional groups: RSH ($n = 3$), RSR ($n = 2$), F ($n = 3$), COOH ($n = 10$) and RCOOR ($n = 6$).

A second assessment of the data was performed by combining similar functional groups. Matrices containing the following functional groups were combined for this inspection: halogens (Cl, F or CF_3), amines (RNH_2, R_2NH, R_3N), acids and esters (RCOOH and RCOOR) and sulfur-containing species (RSH and RSR). The differences observed between the average element recovery from these combined groupings and the average recovery of each element from all of the sample matrices investigated plotted in Fig. 4. All element recoveries for these combined groupings were within ±16% of the overall recoveries. An inspection of the plot in Fig. 4 indicates that, in nearly all cases, the average elemental recoveries by the individual functional groupings are within ±10% of the average recoveries from all of the sample matrices investigated. As expected from the previous assessment, the recoveries for Pt, Hg and Pb in the five sulfur-containing matrices (RSH and RSR) were slightly lower than the overall average recovery for these elements from all matrices investigated (ranging from 10 to 15% lower). The recovery of Sn and Bi in the sulfur-containing matrices and those that contained a cyano group were slightly higher than the overall averages for these elements from all matrices investigated. As noted in the previous comparison above, the total number of matrices investigated that contained either the functional groups RSH, RSR or CN was relatively low ($n = 5$) compared to the total number of matrices investigated in this paper.

The low recovery of Hg in the sulfur-containing matrices is not surprising since thiols are well known for complexing with Hg, and are often used for cleaning up mercury spills. It is suspected that the low recoveries for Hg are due to the formation of a complex not detected at the mercury masses.

3.3. Functionality correlation

A further attempt to understand if the nature of the API has some effect on the recovery of the elements was to conduct a quantitative structure activity relationship (QSAR) analysis on the data obtained. The QSAR analysis compared the recovery of each element with several various structural aspects (as descriptors) of each API investigated, including: sum of atomic polarizabilities; dipole moment in the *x*, *y* and *z* directions; number of rotatable bonds; radius of gyration (relative to the center of mass); molecular weight; molecular density; molecular volume; principal moment of inertia; number of rotational bonds; number of hydrogen bond acceptors; number of hydrogen bond donors; partition coefficient; presence of C=O groups; presence

Table 4
List of individual element recoveries and chemical functionalities present in all sample matrices investigated

Compound	CO	ROR	OH	NH_2	R_2NH	R_3N	COOH	RCOOR	RCON	CN	Cl	F	CF_3	RSH	RSR	As	Se	Mo	Ru	Pd	Cd	In	Sn	Sb	Pt	Hg	Pb	Bi	Ag
1	1	0	2	1	1	3	0	0	0	0	0	0	0	0	0	95	106	102	104	95	101	95	93	97	97	105	95	87	87
4	0	1	0	0	1	4	0	0	0	0	0	1	0	0	0	90	82	98	99	99	85	111	100	101	120	64	135	101	ND
5	2	1	0	0	2	4	0	0	0	0	0	0	0	0	0	101	105	107	102	107	107	102	102	102	104	94	109	99	65
6	0	0	0	0	1	4	0	0	1	0	0	0	0	0	0	97	103	102	94	68	96	98	89	99	96	109	99	94	96
7	0	1	2	0	2	1	0	0	3	0	0	0	0	0	0	80	71	94	96	98	73	100	93	91	112	74	136	99	ND
8	0	0	0	0	0	0	1	0	2	0	0	0	0	1	1	86	94	98	100	123	65	84	100	89	62	79	71	131	ND
9	1	1	3	0	0	0	0	4	1	0	0	0	0	0	0	102	101	105	102	99	105	93	83	102	108	81	95	107	78
11	0	0	0	0	0	0	1	0	2	0	0	0	0	1	0	99	111	103	96	101	106	88	85	92	77	56	122	88	ND
12	0	0	1	0	0	2	0	0	1	0	1	0	1	0	0	98	83	111	114	107	96	96	98	98	88	102	79	92	74
13	0	1	0	0	0	2	0	0	1	0	1	0	1	0	0	83	81	90	101	87	109	103	122	103	98	97	114	98	ND
14	0	0	1	0	0	1	0	0	2	0	1	0	1	0	0	86	84	97	101	89	95	103	84	99	99	94	90	98	ND
16	0	0	0	0	0	2	0	0	2	0	0	0	2	0	0	93	88	98	94	95	110	69	93	91	71	59	86	75	ND
18	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	97	95	97	101	90	100	98	102	100	81	97	92	83	ND
19	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	108	98	101	107	86	114	116	59	112	100	88	116	117	102
20	0	0	2	0	0	0	0	0	1	0	1	0	1	0	0	100	83	90	94	99	96	93	109	98	101	116	97	83	105
21	1	2	0	0	0	0	0	0	0	0	2	0	1	0	0	102	104	101	97	99	98	99	100	100	74	93	97	94	94
22	0	0	0	0	0	2	0	0	4	0	0	0	0	0	0	96	98	95	101	105	101	101	94	98	86	85	96	100	90
23	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	102	99	99	104	114	110	105	74	104	106	94	112	79	99
25	0	0	0	0	2	1	0	0	0	2	0	0	0	0	0	101	97	101	97	93	97	97	96	100	57	96	95	91	103
26	1	1	2	0	0	1	0	0	1	0	0	0	0	0	1	89	95	98	101	107	104	96	105	93	87	93	92	90	101
27	1	0	1	0	1	2	0	0	0	0	0	0	0	0	0	105	96	103	106	108	107	101	81	110	106	103	81	91	103
28	0	1	3	0	1	1	0	0	2	0	0	0	1	0	0	93	108	115	104	111	106	109	108	109	105	128	105	86	92
29	0	0	0	1	2	0	1	0	5	0	0	0	0	0	0	91	90	102	104	105	104	100	103	101	93	87	105	83	94
30	0	1	0	0	0	2	0	1	1	0	1	0	1	0	0	95	101	99	101	90	101	95	80	103	109	92	90	72	94
31	1	1	2	0	0	0	0	4	1	0	0	0	0	0	0	98	87	99	95	97	97	100	98	101	56	88	98	98	ND
32	0	1	0	0	0	3	0	2	1	0	1	0	1	0	0	96	96	98	101	96	100	82	88	98	103	107	99	93	95
33	0	0	0	0	0	0	0	0	5	0	0	0	0	1	0	104	92	102	109	116	105	111	122	106	73	75	94	117	107
34	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	93	101	107	111	112	111	106	116	112	95	63	96	90	94
35	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	113	103	111	101	109	55	92	91	104	103	78	60	122	ND
36	0	3	0	0	0	1	1	0	1	0	0	0	0	0	0	98	110	102	102	98	92	95	79	99	90	57	96	91	102
37	0	0	2	1	0	0	0	0	1	0	0	0	0	0	0	93	88	96	92	132	90	95	98	96	83	136	121	81	101
38	0	1	1	1	0	4	0	1	2	0	0	0	0	0	0	100	96	97	98	112	100	101	80	104	103	86	112	127	96
40	0	1	0	0	1	7	0	0	1	0	1	0	0	0	0	99	99	99	101	96	95	97	80	98	82	75	90	93	99
41	0	1	0	0	2	1	0	0	4	0	0	0	0	0	0	95	92	94	100	109	102	89	94	90	82	62	86	69	75
42	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	92	95	94	87	107	92	78	83	93	85	75	87	88	91
43	0	1	4	0	0	0	0	0	0	0	0	0	0	0	0	92	92	88	82	95	85	62	68	98	132	96	122	106	68
45	1	1	0	0	1	1	0	0	2	0	0	0	0	0	0	79	90	84	70	86	73	53	86	82	82	72	70	99	69
46	0	2	0	0	0	4	0	0	0	1	0	2	0	0	1	97	102	101	100	107	98	96	110	99	98	73	68	136	87
47	0	0	0	0	1	2	0	0	1	0	2	1	0	0	0	87	100	102	100	105	93	96	79	95	106	109	108	75	102
48	0	0	2	0	0	1	1	0	0	0	0	1	0	0	0	92	99	94	92	103	101	67	86	98	76	91	93	97	75
49	0	3	0	0	0	1	1	1	1	0	0	0	0	0	0	100	96	89	89	108	85	84	72	92	94	78	96	93	104
50	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	116	98	98	99	93	95	95	97	97	108	112	112	114	93
51	0	1	0	0	1	3	0	0	2	0	0	0	0	0	0	99	95	100	105	118	99	101	91	102	87	80	106	91	97
52	0	1	1	0	0	0	0	0	2	1	0	0	1	0	0	85	75	99	84	94	100	106	102	107	75	83	118	120	105
53	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	100	98	101	101	104	99	100	108	102	96	87	96	72	99
54	0	0	1	0	0	4	0	0	2	0	0	0	0	0	0	103	103	89	95	107	94	101	93	100	105	109	110	102	100
55	0	1	1	1	0	1	0	0	2	0	0	0	0	0	0	104	99	101	99	94	98	103	102	102	106	95	105	92	92
56	0	1	0	0	0	2	0	0	3	0	0	0	0	0	0	117	128	113	119	122	109	113	128	112	123	138	126	112	105

Table 4 (*Continued*)

Compound	CO	ROR	OH	NH_2	R_2NH	R_3N	COOH	RCOOR	RCON	CN	Cl	F	CF_3	RSH	RSR	As	Se	Mo	Ru	Pd	Cd	In	Sn	Sb	Pt	Hg	Pb	Bi	Ag
57	0	2	0	1	0	1	0	0	0	0	0	0	0	0	0	106	94	123	108	93	122	107	114	116	99	92	90	115	90
58	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	93	95	98	97	112	123	90	88	106	101	91	106	90	ND
59	0	0	0	0	1	0	0	3	1	0	0	0	0	0	0	117	122	53	55	64	57	54	50	50	90	104	107	102	57
60	0	2	4	0	0	0	0	0	0	0	1	0	0	0	0	126	116	104	101	105	102	101	69	106	91	59	89	76	88
61	0	1	1	0	0	2	0	0	2	0	0	0	1	0	0	91	85	99	101	115	94	93	109	98	102	88	102	140	100
Average recovery																98	97	99	98	101	97	95	93	99	94	90	99	97	92

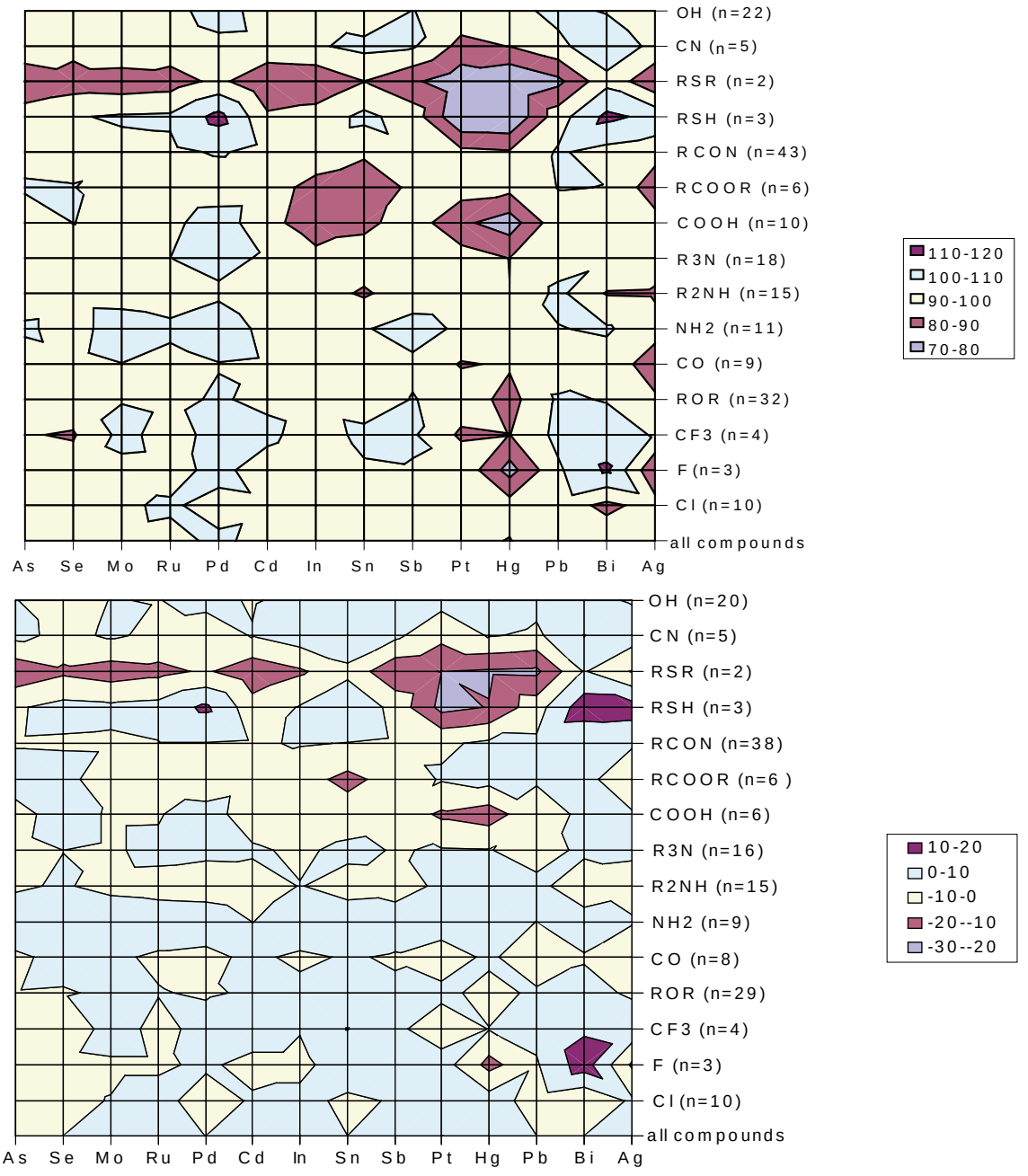

Fig. 3. Comparison plot of the average (%) recoveries for each element of interest vs. the chemical functional group present in the sample matrix (top) and the differences between the average recoveries for the functional group and the average recoveries obtained for all of the sample matrices investigated (bottom).

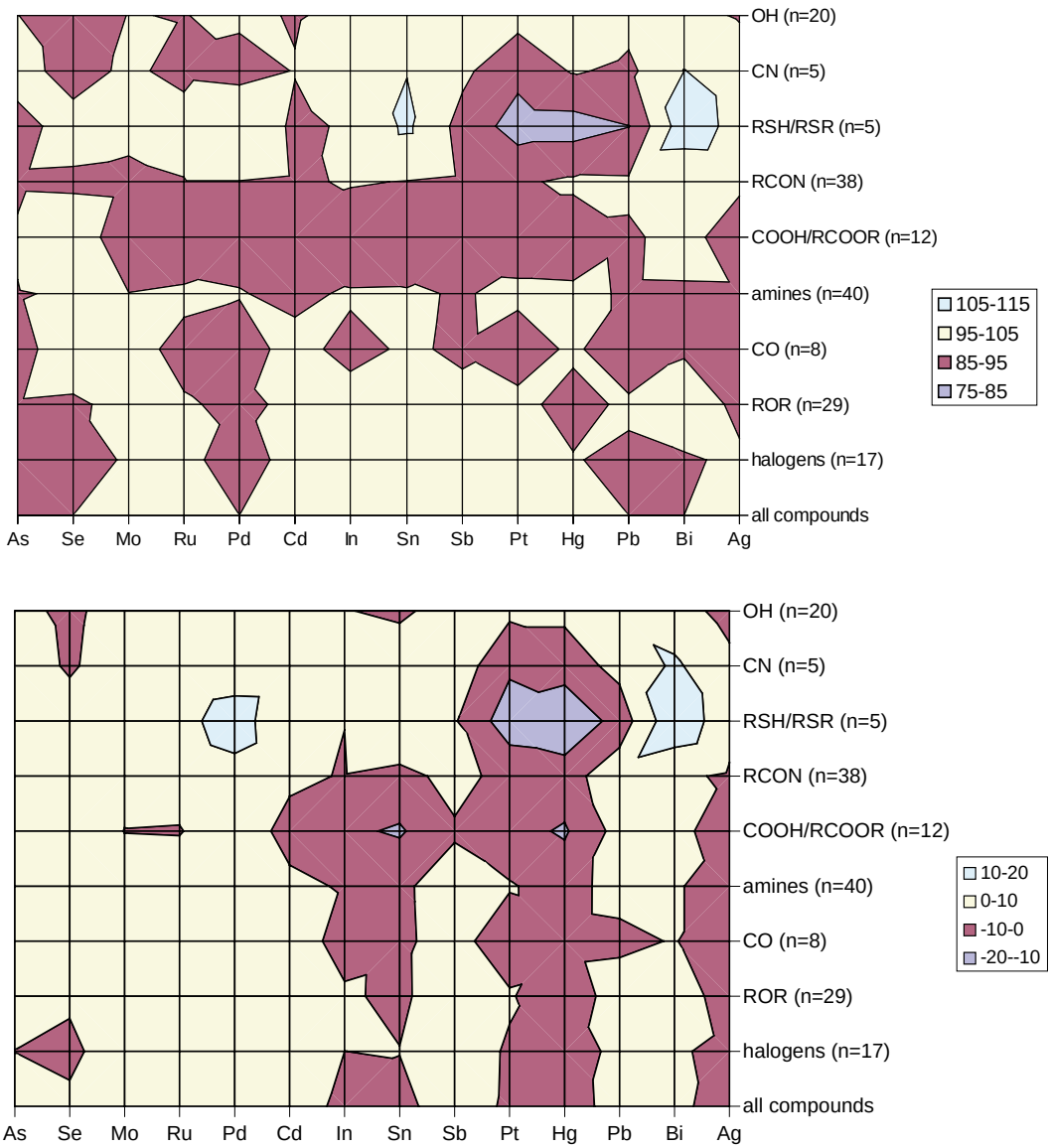

Fig. 4. Comparison plot of the average recoveries for each element of interest vs. similar chemical functional groups present in the sample matrix (top) and the differences between the average recoveries for the functional group subsets and the average recoveries obtained for all of the sample matrices investigated (bottom).

of COOH groups; presence of C–N=C groups; presence of CNH_2 groups; presence of Cl; presence of F; presence of S; and presence of CH_3 groups. Using a genetic function approximation (GFA), the model uses a genetic algorithm to search over the possible models and assigns a lack of fit (LOF) score to estimate the fitness of each model (internal communication with Karthi Nagarajan, 5 April 2001). The QSAR analysis indicated that there was no direct correlation between the compound structure and the elemental recoveries, with R^2 values ranging from 0.449 for Bi to 0.784 for Pt (R^2 values of <0.9 indicate no significant correlation between the two data sets being compared). The QSAR analysis was performed

Table 5
Quantitative structure activity relationship (QSAR) analysis of the elemental recoveries and sample matrices

Element	Using all chemical descriptors (R^2)	Using only functional groups as descriptors (R^2)
Ag	0.655	0.458
As	0.518	0.184
Bi	0.449	0.180
Cd	0.655	0.244
Hg	0.640	0.386
In	0.518	0.416
Mo	0.529	0.480
Pb	0.618	0.447
Pd	0.521	0.485
Pt	0.784	0.568
Ru	0.512	0.522
Sb	0.575	0.489
Se	0.496	0.727
Sn	0.621	0.511

a second time, using only the chemical functional groups as descriptors (internal communication with Karthi Nagarajan, 11 April 2001). Again, the QSAR analysis indicated that there was no direct correlation between the chemical functional groups and the elemental recoveries with R^2 values ranging from 0.180 for Bi to 0.727 for Se. Table 5 lists the correlation values obtained for both QSAR analyses.

The QSAR analysis (Table 5) and the functional group assessment (Figs. 3 and 4) indicate that the described alternative ICP-MS Heavy Metals method is suitable for use with a wide variety of sample matrices. No correlation was observed between the structural characteristics of the sample matrices investigated and the recoveries of the elements of interest, supporting the conclusion that the alternative ICP-MS Heavy Metals test may be used as a general method.

4. Conclusions

The applicability of a general ICP-MS method as an alternative to the compendial methods for the determination of heavy metals has been demonstrated. The ICP-MS screen offers the advantages of a smaller sample size, element-specific information, quantitation, rapid sample throughput and significantly higher recovery of all elements of interest, especially the volatile elements.

The described alternative ICP-MS Heavy Metals test has been utilized in our laboratories for more than 5 years and has been successfully applied to nearly 60 different sample matrices. In addition, the method described has been successfully applied to the ICP-MS instrumentation from two different manufacturers, which further demonstrates the generality of the method. The data presented in this paper demonstrates that the described alternative ICP-MS Heavy Metals test provides a selective and accurate determination of the potential heavy metals present in the sample matrix. The described method shows superior recoveries to the compendial method, especially with regard to the elements Se, Ru, Sn, Sb and Hg, whose recoveries using the compendial method were all <10%. Examination of the individual element recovery data from the different sample matrices investigated and the chemical functionalities present in the sample matrices indicated that there is no correlation between the recoveries of the elements of interest and the sample matrix, demonstrating the applicability of the alternative ICP-MS Heavy Metals test as a general screening method for pharmaceutical compounds. The method can be used to increase the productivity of the laboratory, while providing more accurate and element-specific results.

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