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High-throughput metal screening in pharmaceutical samples by ICP-MS with automated flow injection using a modified HPLC configuration

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ABSTRACT

There is growing pressure in pharmaceutical research and development to increase sample throughput and turnaround time for metal analysis. This need is especially pronounced when a large number of samples must be analyzed to support rapid remediation of metal contamination problems. In this study we describe the utilization of an HPLC–ICP-MS system in automated flow injection (FI) mode for the rapid assessment of metal (palladium, rhodium, chromium, etc.) concentration. The system consists of an HPLC standard or well-plate autosampler, a novel interface (consisting of a desolvating unit, an eluent splitter and a built-in peristaltic pump) and the ICP-MS instrument. This configuration ensures a fully automatic process and is well suited for standardization. The interface can be easily switched to adapt to HPLC eluents or FI introduction for speciation or flow injection analysis. The use of the well-plate autosampler decreases sample injection cycle time and adds flexibility to the system by allowing direct analysis with little or no sample preparation. The FI-ICP-MS method provides a means for high-throughput metal screening with unprecedented speed. Other advantages of the method include automatic analysis of microliter sample volumes, fast inter-sample washout and reduced reagent consumption.

1. Introduction

Inductively coupled plasma-mass spectrometry (ICP-MS) is gaining increasing use and applications in the pharmaceutical industry. This technique has emerged as a powerful analytical technique for quantitative determination of inorganic impurities in raw materials, drug substances and formulated drug products to better meet regulatory requirements. In pharmaceutical process research, it is also a valuable tool for monitoring residual metals in process intermediates and for verifying the effectiveness of purification procedures, thus directing synthetic purification decisions [1–5].

Under increased pressure to accelerate the development process in today's pharmaceutical industry, it is becoming more and more important to improve analytical sample throughput in ICP-MS determinations. For example, removal of the residual metals (palladium, rhodium, ruthenium, etc.) from the drug substances and their intermediates has become a problem of considerable importance, paralleling the growing use of organometallic reagents and catalysts in pharmaceutical synthesis. We recently described a useful approach for rapid evaluation and selection of the most effective adsorbent for remediating metal impurity problems [6,7].

High-throughput metal analysis is also important for tracking and identifying possible metal contamination in the drug manufacturing process, which is a critical issue for product quality. In this case, a large number of randomly selected samples will be collected to provide a statistical representation of the products. The combination of stringent timeline, large number of samples and restricted sample volumes will present an analytical challenge. Again, a high-throughput screening method for a rapid assessment of trace metals will be an invaluable tool for such an investigation.

Sample preparation is typically the bottleneck in ICP-MS determinations. This is especially true when large numbers of samples in organic solutions are to be analyzed. As direct introduction of organic solutions is often hampered by a number of problems such as plasma loading and carbon deposits on the sampler/skimmer cones, the sample solutions normally have to be digested in acidic solutions and diluted to appropriate concentrations. This tedious sample preparation procedure is time-consuming and introduces the risk of contamination or analyte loss during the digestion process. The sample uptake, data acquisition and rinse-out also add up to significant amounts of time with a conventional ICP-MS analysis procedure. Therefore, a high-throughput procedure with minimized sample preparation for both aqueous and organic solutions would greatly increase the sample throughput for ICP-MS determination of large numbers of pharmaceutical samples.

Flow injection (FI) techniques have been well developed as a tool for the automation, acceleration and miniaturization of solution handling in sample preparation and analysis. The combination of

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FI and ICP-MS has also been applied in a variety of fields such as environmental [8], biological [9], geological [10], clinical [11], and industrial research [12–14]. While the need for high-throughput metal analysis in pharmaceutical research and development is of great importance, the application of FI-ICP-MS technique in this area has to date received little or no attention.

In this study, we report the development and use of a method for fast screening of metals in various pharmaceutical samples using FI-ICP-MS. A modified HPLC configuration is applied for easy automation and standardization. The proposed method has been applied to hundreds of samples of various pharmaceutical matrices, such as raw materials, process intermediates and final drug substances. In this work, two case studies, namely fast metal screening in an adsorbent screening test and possible product contamination, will be presented in detail. The determination of palladium (Pd), rhodium (Rh) and chromium (Cr) will be discussed, but the method can also be used for the determination of other metals.

2. Experimental

2.1. Instrumentation

The FI-ICP-MS system (see Fig. 1) employed a modified configuration of an HPLC–ICP-MS system, which was designed for speciation analysis [15]. An 1100 HPLC system (Agilent Technologies, Wilmington, DE, USA) was coupled to an Agilent 7500cs ICP-MS through a custom-built interface. The ICP-MS was equipped with an Octopole Reaction System (ORS), which used helium or hydrogen as a collision or reaction gas to eliminate matrix or plasma based polyatomic interferences. An HPLC binary pump with a vacuum degasser was used for the introduction of a continuous carrier stream, and a thermostated HPLC regular autosampler or a microplate autosampler was used for sample injection. No HPLC column was used and the outlet was connected to an interface before the sample was transferred into a desolvation unit. Deionized water with a flow rate of 2.0 mL/min was used as a carrier stream. The injection volume for each sample is normally 2 μL. But larger volumes can be used for samples with lower metal concentrations or when isotopes of poor sensitivities are to be determined.

The interface consisted of a PEEK Tee (0.50 mm, Upchurch Scientific, Oak Harbor, WA, USA) which was served as an eluent splitter, an Aridus Desolvation Sample Introduction unit (CETAC Technologies, Omaha, NE, USA) and the ICP-MS built-in peristaltic pump. It was similar to that described before for the HPLC–ICP-MS experiment [15] except that no HPLC column was used and the eluent split ratio was adjusted according to the flow rate of the carrier stream. A portion of the carrier stream and the injected sample solution was nebulized into a heated PFA spray chamber using a PFA microconcentric nebulizer, and transported to a heated microporous PTFE tubular membrane. The membrane can be heated up to 160 °C so that any solvent with a boiling point of less than 160 °C will be changed into a solvent vapor. The solvent vapor passed through the membrane and was removed by a stream of argon gas, while the analyte continued through the tube to the ICP-MS. Through this mechanism the desolvating unit enabled the handling of both organic and inorganic solutions without plasma loading. "Chromatographic" data analysis was made by using the Agilent Plasma Chromatographic software. The instrument settings were checked daily and optimized when necessary.

For purposes of comparison, some samples were also analyzed using a conventional method [1] with an Elan 6000 quadrupole ICP-MS (PerkinElmer, Inc., Shelton, CT, USA).

Operating conditions of all the instrumentation are summarized in Table 1.

2.2. Sample collection and preparation

2.2.1. Adsorbent screening test for residual metal removal

An adsorbent screening kit which includes 24 stockpiled microcentrifuge tubes containing 50 mg of each of a variety of different adsorbents was used for the residual metal removal study. More detailed description of the adsorbents can be found elsewhere [7]. An in-process pharmaceutical intermediate with an elevated Pd concentration was dissolved in ethanol and then treated with the kit. After mixing and shaking with the adsorbents, the supernatant in each tube was transferred to a 2-mL HPLC vial, which was then placed into the autosampler tray for the determination of Pd by FI-ICP-MS.

A calibration curve was established by injecting and measuring three standard solutions (0.5, 1.0, and 2.0 μg/mL of Pd, respectively) which were freshly prepared in ethanol. The samples were injected in a same sequence with the standard solutions. The obtained FI-ICP-MS peaks were integrated using the Agilent Plasma Chromatographic software; all the samples were quantified by

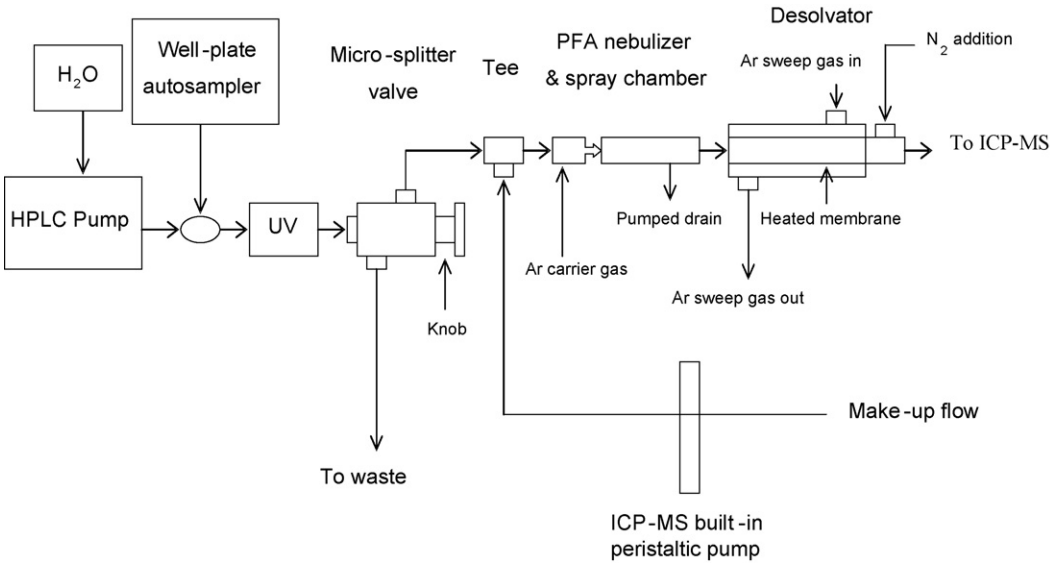

Fig. 1. Schematic diagram of the FI-ICP-MS system.

Table 1
Operating parameters for the instruments used in the experiments.

Aridus conditions	
Sweep gas flow	72 mL/min
N2 gas flow	10 mL/min
Carrier gas flow	0.88 mL/min
Spray chamber temperature	110 °C
Desolvator temperature	160 °C
Agilent ICP-MS conditions for FI-ICP-MS	
RF power	1100 W
Plasma gas flow	14.9 L/min
Auxiliary gas flow	0.90 L/min
Sampling depth	13 mm
Peristaltic pump speed	0.22 rps
Acquisition mode	Time resolved analysis
Integration time	1.00 s
PerkinElmer ICP-MS conditions for Pd analysis with conventional sample introduction	
Power	1300 W
Plasma gas flow	15.0 L/min
Auxillary gas flow	1.0 L/min
Nebulizer gas flow	0.80 L/min
Sample introduction	Cross-flow nebulizer with Scott spray chamber
Scanning mode	Peak hopping
Dwell time	100 ms
Sweeps per reading	10
Readings per replicate	3
Number of replicate	3
Isotopes monitored	106, 108, 110

comparing the peak areas of the samples with those of the standards.

For the purpose of comparison, the above samples were also digested/diluted with 80% (v/v) nitric acid and analyzed by the PerkinElmer ICP-MS using the conventional sample introduction method [1].

2.2.2. Investigation of possible Cr contamination in filled pharmaceutical products

Another FI-ICP-MS case study was carried out as part of an investigation of possible Cr contamination in filled pharmaceutical products. We were presented with a situation where discolored liquid was observed in the meniscus of filling pumps during the manufacturing process of a product. The root cause was identified as pump wear. The particles from the discolored liquid were identified as chromium (III) oxide. A statistical analysis showed that analysis of 87 randomly selected sample vials, from a total of 460, should undergo Cr analysis to provide 95% confidence that at least 10 vials filled by the affected pump will be represented among the 87 vials tested.

For FI-ICP-MS analysis, the septa of the product vials were removed to eliminate any potential problem of blocking the HPLC injection needle. The vials were then put on the 15-vial tray, placed into the regular HPLC autosampler, and analyzed.

For quantitation, any sample with a significantly high Cr concentration was acidified with nitric acid to make the final acid concentration 1%. Standard Cr solutions of 10, 20 and 50 ng/mL were prepared by serial dilution of a 1000 μg/mL stock solution with 1% nitric acid, and were then injected together with the selected samples in the same sequence.

2.3. Reagents

Stock standard solutions with a concentration of 1000 μg/mL for each individual metal (Pd, Rh, Cr) were purchased from High-Purity Standards (Charleston, SC, USA). The Rh standard was used during method development. Concentrated nitric acid (70%, trace metal grade) was purchased from Fischer Scientific (Fair Lawn, NJ, USA). The deionized water used in the experiments was prepared by passing water through a Hydro Ultrapure water system (Hydro Service and Supplies, Garfield, NJ, USA). All the drug substances and their intermediates used in this study were obtained from Merck Research Laboratories.

3. Results and discussion

3.1. System optimization

3.1.1. The interface

Most samples analyzed in support of pharmaceutical process development are prepared in organic solutions. However, direct injection of an organic solution into the ICP-MS will lead to increased solvent loading in the plasma, which will result in a number of problems including unstable or even extinguished plasma, decreased sensitivity, carbide polyatomic ion interferences and carbon deposits in the ICP sample introduction area. To overcome these problems, a unique interface coupled with an Aridus Desolvation Sample Introduction unit has been developed in our laboratory with increased sensitivity [15]. The interface was initially developed for HPLC–ICP-MS applications, but we found that with slight modifications (Fig. 1) it was also perfectly suited for flow injection analysis.

For better quantitation, sharper peaks are preferred in this analysis. Therefore the flow rate of the carrier stream should be kept at a maximum possible value. In Fig. 1, the carrier stream from the LC autosampler outlet reaches a Tee, which serves as a splitter, before it is connected to the peristaltic pump tubing on the ICP-MS. The split ratio is determined by the diameter and length of the tubing, and by the flow rate of the peristaltic pump. By using an appropriate argon carrier gas flow rate (0.9 L/min) and maximizing the peristaltic pump speed, a carrier stream flow as high as 2.0 mL/min can be used in the system without overloading the membrane desolvator.

Plasma and the ion lens settings required major re-optimization when the coupling was used for the first time, because the optimized parameters were largely different from those used for conventional wet sample aerosols. Sensitivity is improved with the Aridus by enhancing analyte transport efficiency and reducing solvent loading to the plasma. The addition of a small amount of N_2 to the carrier gas also resulted in significant enhancement in sensitivity for all the elements investigated, the mechanism of which is still unclear. Compared with conventional pneumatic nebulization, the sensitivities of $^{7}Li^{+}$, $^{89}Y^{+}$, and $^{205}Tl^{+}$ used for tuning were enhanced by more than 10 times with the Aridus. The degree of sensitivity enhancement is dependent on the isotope analyzed and the solvent used. No visible carbon built-up was observed after prolonged use, thanks to the use of the Tee splitter which limited the amount of organic solvent entering the Aridus. The addition of oxygen, though recommended by CETAC, seemed not needed when an appropriate nebulizer gas flow rate was selected. Operating without oxygen addition was advantageous because problems such as the increase of the oxygen-containing polyatomic ion interferences and accelerated degradation of the sampler and skimmer cones could be avoided.

The improvement in sensitivity with the membrane desolvation may be beneficial to FI-ICP-MS analysis in many research applications. However, sensitivity is generally not a problem for most of the samples analyzed in our research group, as the metal (Pd, Rh, Ru, etc.) concentrations are usually in the high ppb to low ppm range. On the contrary, there is often a need to reduce the signal in order to avoid broad peaks during analysis. This can be easily accomplished by reducing the sample injection volume. Sensitivity of the measured isotopes can also be decreased by more than 10 times by reducing the amount of N_2 used in the desolvation

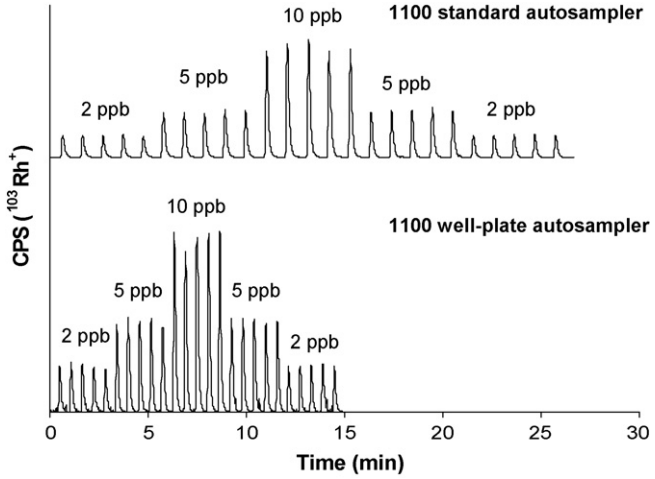

Fig. 2. Comparison of injection cycles of Agilent 1100 standard and well-plate autosamplers for the determination of Rh standards in acetonitrile (2, 5 and 10 ng/mL, respectively) using FI-ICP-MS (injection volume: 2 μL).

unit. Another convenient way of suppressing signal is to introduce a collision gas of helium into the system, the amount of which is normally adversely correlated to the ICP-MS signal.

The ChemStation and Plasma Chromatographic software integrated with the Agilent HPLC and ICP-MS makes the FI-ICP-MS analysis a fully automated process, and this configuration is well suited for standardization. The interface can also be easily modified to switch between speciation analysis and flow injection analysis.

The proposed method has been successfully applied to hundred of samples with no major issues identified, indicating that this configuration is well suited to an easy and robust setup of high-throughput metal analysis.

3.2. Comparison of autosamplers

The Agilent 1100 HPLC standard autosampler provided adequate sample introduction for flow injection analysis. A typical FIAgram of five injections of Rh standard solutions (2, 5, and 10 ng/mL in acetonitrile) is illustrated in Fig. 2. Relative standard deviations of 11 replicate injections of the 2 ng/mL standard were 3.6%. The limit of detection (LOD) for Rh analysis, defined as three times of the standard deviation of the 11 measurements, was found to be 0.15 ng/mL at an injection volume of 2 μL. Since rhodium is a mono-isotopic element, slightly higher LODs can be expected for other catalyst metals.

Though the standard autosampler provided adequate performance for this application, a higher sample throughput can be achieved by shortening the injection cycle time. Therefore an Agilent well-plate autosampler was tested for sample injection. The injection cycle time was made as short as possible (~25 s) for high sample throughput as long as good peak shape was maintained for quantitation.

A comparison of sample injections of the same set of Rh standard solutions using these two autosamplers can be found in Fig. 2. It can be noted that the well-plate autosampler has achieved another 45% increase of sample throughput thanks to its shorter injection cycle. For samples with higher metal concentrations, however, a longer interval between sample injections may be needed to avoid sample carryover and to maintain a stable baseline.

Another advantage of using the well-plate autosampler is that samples in the well plates prepared by high-throughput experimentation researchers in chemistry or separation science studies can be directly analyzed, without need for sample format transfer or manipulations. Therefore increased flexibility can be added to the system for higher sample throughput.

Table 2
Comparison of results by ICP-MS analysis with flow injection or conventional sample introduction (X_{imp} is the percentage of Pd left in the solution after adsorbent treatment).

Sample ID	Adsorbent	Flow injection		Conventional	
		Pd concentration (μg/mL)	X_{imp} (%)	Pd concentration (μg/mL)	X_{imp} (%)
1	Silica	24	35	36	47
2	Smopex 105	29	42	39	51
3	Smopex 111	50	72	59	78
4	SN-Bio	14	20	23	30
5	SP207	32	46	46	61
6	Thiol-3	9	13	12	16
7	GL-961	28	41	34	45
8	HP20	33	48	45	59
9	MP-TMT	4	6	2	3
10	Nuchar RGC	18	26	24	32
11	Nuchar TAC	54	78	59	78
12	Nuchar 400	17	25	24	32
13	C-933	24	35	39	51
14	C-941	14	20	28	37
15	C-943	27	39	48	63
16	Calgon ADP	32	46	39	51
17	Darco G60	38	55	49	64
18	Darco KB-G	24	35	27	36
19	Alum Oxide	43	62	58	76
20	Amb XAD16	42	61	67	88
21	AquaGuard	11	16	16	21
22	C18	58	84	75	99
23	C-905	27	39	31	41
24	C-908	27	39	34	45
Untreated	–	69		76	

3.3. Comparison of results from FI-ICP-MS and conventional ICP-MS

For comparison purpose, a set of samples was analyzed for Pd using both FI-ICP-MS and conventional ICP-MS. This set of samples was prepared by dissolving a pharmaceutical intermediate in ethanol and then treating with 24 different adsorbents [7]. The adsorbents used for the test are listed in Table 2. Supernatants from the treated samples and an untreated sample were used for the FI-ICP-MS analysis. The FIAgram of the analysis is shown in Fig. 3. The same set of samples was then digested/diluted 1000-fold with 80% (v/v) nitric acid and analyzed with ICP-MS using conventional sample introduction [1]. A comparison of the results can also be found in Table 2.

It can be observed that results from the two methods are largely consistent with each other. The numbers from the flow injec-

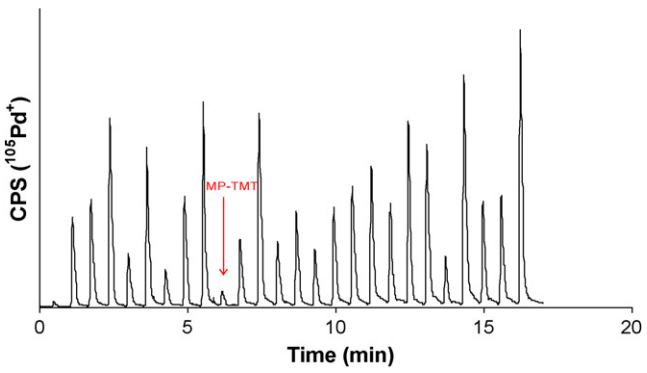

Fig. 3. FIAgram obtained with the FI-ICP-MS system for Pd analysis in an adsorbent screening test.

tion method are slightly lower than those from the conventional method, probably a result of a more severe matrix effect from the undigested samples. In addition, the precision of the flow injection approach, having a nature of time resolved analysis, is generally not as good as that of the conventional method. Although approaches such as internal standardization or standard addition may compensate for matrix effect and repetitive sampling may improve precision, no attempt was made to apply these approaches considering that high sample throughput is the first priority of this research. It can be seen from Fig. 3 that a "hit" signifying the best efficiency in Pd removal from the sample solution was identified within 17 min. As will be discussed in the next section, what really matters in the adsorbent screening test is the relative ratio of the metal concentrations between the treated and untreated samples, instead of the absolute concentrations.

A minimum volume of 2 μL can be injected using the FI-ICP-MS system. Compared with conventional ICP-MS, this has the advantage of automatic analysis of microliter sample volumes, fast inter-sample washout and reduced reagent consumption.

3.4. Applications of FI-ICP-MS

With minimum sample preparation and unprecedented speed, this FI-ICP-MS method is well suited for fast metal screening of large amount of samples. Two examples of potential applications are discussed below.

3.4.1. Adsorbent screening test for the removal of residual metals

Residual metal impurities can be removed from pharmaceutical intermediates or APIs *via* selective adsorption, provided that an adsorbent with suitable selectivity and capacity can be identified. A microtube screening approach has been developed for surveying the selective adsorption of metal impurities by a variety of different process adsorbents [6,7].

As reported previously [7], the selectivity (α) is estimated from the measurement of the concentration of product (*e.g.*, an intermediate or API) and impurity (*e.g.*, metal) using the equation shown below.

$$\alpha_{\text{apparent}} = \frac{(1 - X_{\text{imp}})/X_{\text{imp}}}{(1 - X_{\text{prod}})/X_{\text{prod}}}$$

where α_{apparent} is the apparent selectivity, X_{imp} the percentage of the impurity free in the solution, X_{prod} is the percentage of the product free in the solution.

Any means to enable a rapid selection of the most appropriate adsorbent and condition for a given purification task would be ideal. However, the time spent on metal analysis of 25 samples with a conventional method has made a high-throughput evaluation impossible.

To this end, FI-ICP-MS has been applied to numerous adsorbent screening tests in our lab. Typically 1 untreated sample and 24 samples treated with different adsorbents are used for the screening test. An example of the test is shown in Fig. 3. One adsorbent, MP-TMT, can be quickly identified as a promising lead for the removal of Pd and the values of X_{imp} (also listed in Table 2) for all the adsorbents can be calculated. As mentioned earlier, the ratio between $(1 - X_{\text{imp}})$ and X_{imp} is used for the calculation of apparent selectivity (α_{apparent}), so the actual metal concentrations are of less importance in this application. It can be noted that the X_{imp} values obtained from the FI-ICP-MS and the conventional method are in consistent with each other for the large majority of the samples. This is more than adequate for selecting an appropriate adsorbent in a high-throughput analysis.

As product loss may occur with adsorbent treatment, measurement of the product (*i.e.*, the intermediate) recovery is equally important in the calculation. This is a much easier job and the data can be easily obtained using flow injection analysis with an HPLC equipped with an UV or MS detector [7].

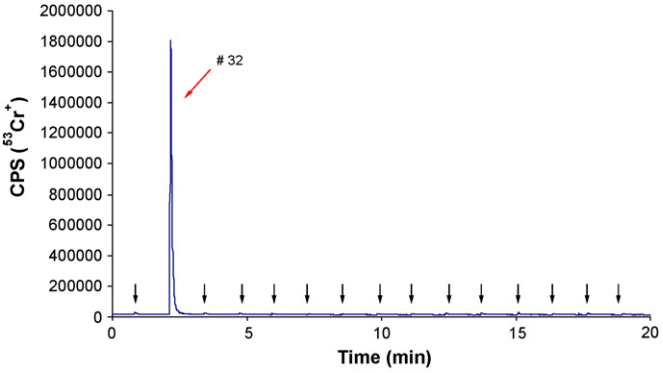

Fig. 4. FIAgram obtained with the FI-ICP-MS system during a quick investigation of possible Cr contamination.

Initial tests were carried out to combine the two tests into one by flow injection with UV detection followed by ICP-MS detection. However, this approach does not seem to be applicable to most of our samples, primarily due to a large difference in sensitivity between the UV and ICP-MS detection. Furthermore, an HPLC column is often needed so that the required product can be separated before its concentration is determined. This approach would result in difficulty in the subsequent ICP-MS detection. Therefore it was decided that the two flow injection measurements be carried out separately.

Combining results from the two measurements, the selectivity values for all the adsorbents can be calculated and the most appropriate adsorbents for Pd removal can be identified.

3.4.2. Investigation of possible metal contamination

Trace metal contamination may occur in pharmaceutical manufacturing process, which is a critical issue for product quality. In case of an investigation, a large number of samples must be analyzed in order to provide a statistically significant representation of the "worst case" contamination scenario. A recent example from our laboratories illustrates the suitability of this screening approach with FI-ICP-MS. Quantification of Cr in a large number of filled vials was critical in determining if chromium oxide was able to migrate from the pump meniscus into filled product and if so, at what level was Cr present in the filled product. A total of 87 selected filled vials, each containing about 0.7 mL of water, were subjected to Cr analysis in a very tight timeline.

Conventional ICP-MS methods are not practical for these samples, since large expenditure of labor and time alone would make it impossible to meet the deadline. In addition, dilution of the samples to a required volume (at least 3 mL for each analysis) may lead to Cr concentrations below the detection limit.

The FI-ICP-MS method was found to be perfectly suitable for this application. No sample preparation was needed. Compared to the conventional ICP-MS method, the FI-ICP-MS method achieved a 3-fold increase in sample throughput and improved reportable limit of detection without the need of sample dilution. Since only 2 μL of each sample was needed for injection, this method was particularly ideal for samples with very limited volumes.

Part of the obtained FIAgram is illustrated in Fig. 4, which clearly indicates that one sample (# 32) has significantly higher Cr concentration than the others. The sample was then quantified by comparing its peak area with those of the blank and the standard

solutions. Cr concentration in this sample was found to be 12 ng/mL. The results of this analysis provided critical evidence for a further investigation.

4. Conclusions

The modified HPLC configuration is perfectly suited for automated flow injection for sample introduction of both aqueous and organic solutions and the novel interface enables its easy coupling with ICP-MS. The FI-ICP-MS method has provided a means for high-throughput metal screening of large amount of samples and has been successfully applied to metal analysis in adsorbent screening tests and in contamination investigations. The utilization of the method will provide a new level of automation and sample handling for metal analysis in pharmaceutical research and development.

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