Organic Process Research & Development **1999**, *3*, 44–52

Cleaning and Decontamination of Potent Compounds in the Pharmaceutical Industry

Ioannis I. Valvis* and William L. Champion, Jr.[†]

DuPont Pharmaceuticals Company, Chambers Works, PRF (S-1), Deepwater, New Jersey 08023

Abstract:

One of the significant issues encountered when handling potent compounds is the ability to clean contaminated surfaces to a safe and operable level. Such a level, whether driven by good manufacturing practices (GMP) or industrial hygiene (IH) guidelines, can be low enough to be a challenge. In dealing with it, emphasis must be placed on (i) establishing meaningful criteria for cleanliness, (ii) formulating a rationale for the design of a cleaning procedure, (iii) developing a deactivation ("kill") solution, when possible, and (iv) developing a reliable analytical scheme. This paper discusses the above topics in detail as they apply to various operations in the pharmaceutical industry. Furthermore, two cases involving the cleaning of a nondegradable cytotoxic compound following a manufacturing campaign, and the cleaning of an anti-thrombotic compound following a laboratory spill incident, are presented.

Introduction

The development of highly active drug substances, commonly referred to as "potent compounds", has become a common trend in today's pharmaceutical industry. Handling such compounds has presented manufacturing with special challenges, including "containment", "cleaning", and "decontamination". Two classes of compounds in particular, anti-cancer and anti-thrombotic agents, are of the greatest concern due to their respective toxic or pharmacological potential. Due to their low-dose therapeutic profile, these classes of compounds can present serious risk of exposure to humans in a work environment and can lead to product cross-contamination in manufacturing. For this reason, great effort has been made by companies to acquire or develop the necessary technology for the cleaning and/or deactivation of such compounds. Since supporting such efforts can require significant resource commitment, the need for better understanding of this emerging challenge is great. The subject of containment has been discussed by one of us (Valvis) in an earlier publication[1] and by others.[2−4] This paper's objective is to provide some fundamental insight into the cleaning and decontamination aspects of manufacturing, especially in the area of potent substances. The present topic has also been discussed by others in the field, but primarily as it pertains to specific compounds with mutagenic potential.[5−7]

Understanding the Issues and Challenges. Before continuing, it is essential that the definitions for some of the key terms used in this paper, such as *cleanability*, *decontamination*, and *non-detect levels*, are given. Cleanability describes the extent to which a surface can be cleaned so that it can be operated safely and produce a product of acceptable quality. Typically, the term non-detect (ND) is used to measure cleanliness relative to the limit of analytical quantitation (LOQ). Decontamination is a more absolute term, and it describes the complete elimination of contaminants via a deactivating ("kill") solution. When deactivating agents are not available, it is very important that an adequate safety margin be built into the cleanliness criteria to ensure personnel safety and to prevent cross-contamination (carryover to the next product). Unfortunately, no standards for selecting safety margins or establishing cleanliness criteria exist in the industry. As a result, each company develops its own guidelines on cleaning requirements for the various classes of compounds. One such approach uses acceptable daily intake as the basis for such determinations.

Acceptable daily intake for humans is normally calculated by applying a hazard risk factor to toxicological and pharmacological data which establish a "no-effect level" for humans. Typical hazard risk factors range between 1/100 and 1/10000 and usually vary in an inversely proportional manner relative to the potency of a substance and completeness of toxicity/pharmacology data available. During the early phases of development, the only available data come from small animal toxicological studies or other products having similar pharmacological profiles. Dependent on the nature of the drug, a risk factor of 1/1000 is commonly applied to no-effect or low-effect dose levels, to produce an adequate margin of safety. Later in drug development, as more scientific data become available, it is sensible that risk factors be reevaluated for appropriateness and to minimize any unnecessary cleaning efforts and costs. In general, the safety margins are tightened or relaxed only within 1 order of magnitude based on observed toxicity, pharmacokinetics, metabolism, and potency in humans. In calculating cleaning requirements during the early phases of development, companies in the industry tend to make use of formulas

[†] W. L. Champion, Jr., is currently employed by Chiral Technologies Inc.

(1) Valvis, I. I. Manufacturing of Potent Compounds at Pilot Scale, 46th CPI Exposition, New York, December 1995.
(2) Nichols, J. E. Chemical Containment in Bulk Pharmaceutical Plants. *Pharm. Eng.* **1991**, *11* (3), 9−13.
(3) Larkin, R. G.; Monk, R. E. Development of Primary Containment in Bulk Pharmaceutical Facilities. **1993**, March/April, 9−16.
(4) Zeller, A. O. Cleaning Validation and Residual Limits: A Contribution to Current Discussions. Pharm. Technol. **1993**, October, 70−80.
(5) Misner, J.; Kennedy, J.; Biggs, W. Pergolide: Process Design Challenges of a Potent Compound. *Chemtech* **1996**, November, 2−7.
(6) Benvenuto, J.; Connor, T. Degradation and Inactivation of Antitumor Drugs. J. Pharm. Sci. **1993**, *82*, 988−991.
(7) *Cytotoxic Drug Safety Cabinets—Installation and Use*; AS 2639-1983; Standards Association of Australia: North Sydney, NSW, Australia, 1983.

10.1021/op980181d CCC: $18.00
Published on Web 12/17/1998

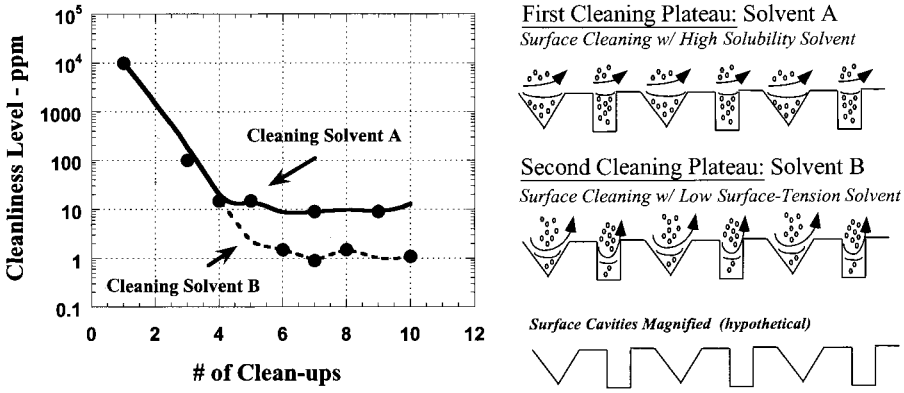


Figure 1.

which are based on representative cleaning scenarios and on corporate guidelines for personnel exposure. A common practice observed in most companies today involves the use of three basic formulas: (i) the 10 ppm formula, (ii) the acceptable daily intake formula, and (iii) the 1/1000 of therapeutic level formula.

The 10 ppm Formula. This formula allows no more than 10 ppm of product A into the next product's (B) maximum potential daily dose. Given the production scale of product B, the acceptance level is calculated as milligrams of product A per liter of cleaning solution.

The Acceptable Daily Intake Formula. This formula allows no more product A into product B than what the acceptable daily intake dictates. The acceptable daily intake of A can sometimes be estimated from an 8-h time-weighted, acceptable workplace exposure limit (AWEL). In this case, the acceptable daily intake would equal the exposure limit times 10 m^3, which is the conventional volume of air inspired during an 8-h work shift. However, considerable caution should be exercised when using this approach to ensure that risk and uncertainty factors used to determine workplace exposure limits are appropriate to cleaning validation determinations. Calculation of the acceptance level requires knowledge of product B's production scale.

The 1/1000 of Therapeutic Level Formula. This formula allows no more than 1/1000 of product A's minimum therapeutic daily dose into product B's maximum daily dose. Calculation also requires knowledge of product B's production scale.

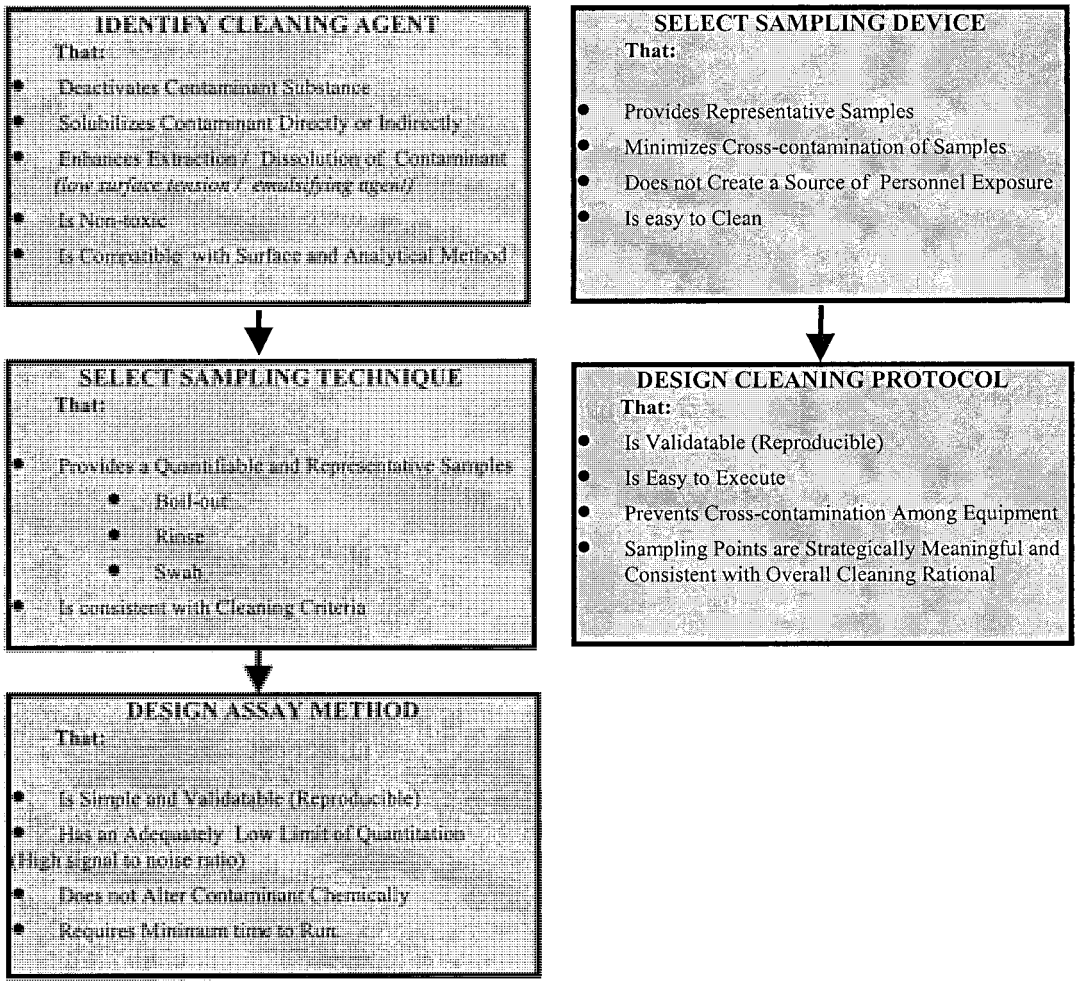
Depending on data availability, cleaning criteria are frequently based on the formula that results in the lowest number—most typically the last one described above. When dealing with some suspected potent compounds very early in the development process, cleaning criteria could be derived from default workplace exposure limits, such as 10 $\mu g/m^3$ or 10 ppm, unless contraindicated by expected effects of the compounds (e.g., mutagenicity, developmental toxicity, etc.).

It is apparent from the above that the more active a substance, the lower the cleaning criteria would be, and the larger the effort and cost of maintaining product quality and personnel safety would be, making the cleaning and decontamination a serious concern and a major cost contributor in manufacturing.

Formulating a Rationale for the Design of a Cleaning Procedure. Cleaning after use of potent compounds requires a considerable amount of effort and time from both analytical and processing personnel. In the case of pilot plant operations, a cleaning protocol with poor rationale may lead to bottlenecking in development, while in the case of bulk production, it may cause scheduling conflicts and increased overhead costs. Of course, the situation is a lot simpler when a "kill" solution is available. When this is not the case, the answer to successful cleaning becomes a matter of developing an effective and efficient cleaning protocol. The rationale behind the selection of cleaning solvents, the choice of an analytical method and the design of a cleaning procedure must be developed carefully before processing begins. In addition, the criteria for accessing cleaning as successful must be agreed in advance and be well defined.

Three of the most common procedures performed in order to obtain a final sample for establishing cleanliness are *boil-outs*, *rinses*, and *swabs*. A boil-out involves the refluxing of a solvent inside a closed vessel system in order to clean its interior surfaces and produce a representative sample. The cleaning effectiveness of a boil-out relies on the combined actions of dissolution, mixing shear and vapor extraction, all resulting in an exponential dilution cleaning profile (see Figure 1). A rinse sample is obtained when a boil-out is not feasible (i.e., cleaning piping and miscellaneous portable equipment). In such cases, the use of clean-in-place (CIP) spraying or misting nozzles facilitates the cleaning of hard-to-access areas, but never as efficiently as vapor cleaning. A swab sample is obtained by wiping a surface with solvent-moistened cotton gauze, and it is used to grossly quantitate the presence or the absence of a contaminant. The calculation of a cleanliness level for a piece of equipment is based on the assumption that all surfaces are uniformly contaminated. In reality, such an assumption is not always valid, since neither the finish of the contacting surfaces (shape, roughness) nor the nature of the contact with the contaminant (liquid, solid, vapor) is the same everywhere. This is especially true with complex wet-processing equipment (reactors, filters-dryers, etc.) and less so with formulation-type equipment, whose surfaces have a more uniform and finer finish and can be easily accessed for cleaning. Some other factors that affect the accuracy of swab calculations

Scheme 1. **Road map for developing an effective cleaning procedure**

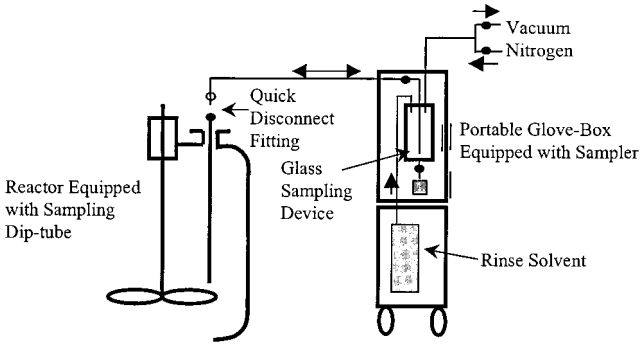


include the technique of applying the swab and the method of analyzing the swab sample (extracting swab-gauge and assaying extract). Variabilities in these operations can reduce significantly the overall recovery of the swabbing process, producing nonreliable and nonreproducible results. To minimize such variabilities, all calculations should be based on the average of a large number of randomly taken samples, and results must be viewed in a "ball-park" magnitude manner; otherwise, erroneous conclusions may be drawn. For example, errors frequently arise from using the swab testing approach to validate boil-out-based cleaning results. In general, it is recommended that swab testing be used to perform only qualitative assessments (absence or presence of a contaminant) of cleanliness, as in decommissioning operations. Decommissioning is the process of verifying the *absolute* cleanliness of equipment or work area prior to its release for the manufacturing of other compounds. When a more quantitative assessment is unavoidable, a margin of at least ±50% of the action limit (cleanliness criterion) should be allowed to account for variabilities in recovery.

To summarize, the extraction efficiency, effectiveness, and practicality of boil-outs is superior to that of swab testing when dealing with larger processing equipment. Since boil-outs result in exponential dilution cleaning profiles, the results from two consecutive boil-outs are sufficient to validate cleanliness. The use of a rinse to obtain a final sample is not recommended for closed-type equipment, since all products contacting surfaces may not be accessible. The technique of swab testing has many limitations associated with the ability to obtain samples that reflect the true state of cleanliness of the entire surface of a process equipment. Also, it exhibits a high degree of variability. To estimate such variability, it is suggested that, prior to implementing a swab test, the method be developed using samples ("coupons") of surfaces similar to the ones that will be actually tested for cleanliness. This allows not only estimating percent recovery but also optimizing the analytical method in terms of solvent compatibility and chromatographic resolution capacity.

The block diagram in Scheme 1 provides a road map for the development of an effective cleaning procedure. The selection of cleaning agents is based on their ability to enhance the dissolution and extraction of contaminants from the crevices and microcavities of a surface. When cleanability becomes limited, i.e., a plateau in the concentration profile is reached, then another cleaning agent with different properties must be introduced (see Figure 1). Such properties include lower surface tension, higher solubility, and surfactant characteristics. Due to its broad solubility strength and low cost, a solvent such as methanol is frequently used for this purpose. Methanol does not interfere with conventional reversed-phase chromatographic methods and is compatible

Scheme 2. **Sampling of potent compounds**



with most surfaces. Surfactants can also be used, but they have been frequently found to interfere with the assay method. With regard to the sampling technique, the choice is determined by the type of process equipment involved. In the case of reactors, a sample of refluxed solvent (a boil-out), obtained via a sampling tube, is very common. It is the authors' experience that a portable sampling device, attached to a dip-tube on the reactor via quick disconnect fittings, offers two additional benefits: cleanability and minimization of personnel exposure. Scheme 2 shows a schematic of a portable sampling device: the unit consists of easy-to-disassemble components and is contained. The sampling device and associated lines are flushed with clean solvent and nitrogen before disconnecting.

When dealing with multireactor setups, it is recommended that reactors be divided into smaller groups, segregating the most contaminated reactors from the least contaminated ones. By doing so, cleaning efforts are minimized and the migration of contaminants is controlled. Furthermore, trouble-shooting, in the event that cleaning problems arise, becomes easier. A typical reactor setup includes a reactor equipped with condenser, a receiver, and a pump. For such a case, a cleaning protocol involves performing a number of rinses and boil-outs and circulating solvent from one vessel to another, maintaining a direction of cleaning from the least contaminated vessel to the most contaminated one. Circulation of cleaning solvent through the vessels and their associated piping should be performed only after compatible levels of cleanliness have been demonstrated for each vessel. When a boil-out is not practical, a rinse is applied typically via 360° rotating CIP nozzles. CIP nozzles attached on flanged ports of the process equipment ensure better cleaning and help to maintain containment during the cleaning process.

The development of an assay method that can detect contaminants at a low ppm level can also be very challenging. Critical issues such as the compatibility of the cleaning solvent(s) with the solvents used in the analytical methods must be addressed. Also, analytical method parameters such as mobile-phase solvent ratios and injection volume-to-sample concentration ratios must be carefully tuned so that the desired assaying sensitivity and reproducibility are achieved. From an operational standpoint, attention must be paid to the cleaning instructions and the recommended techniques so that the entire cleaning effort is effective as well as economically sensible.

Typical cleaning protocols include the following four stages: gross cleanup, surface passivation, exponential cleaning, and microcleaning.

I. Gross Cleanup to a Visually Clean Level. In the case of reactors, this cleanup involves rinsing via a CIP nozzle followed by a boil-out; all other equipment is rinsed twice with a cleaning solution. In the case of exterior surface cleanup, rinsing is replaced by low-pressure misting followed by a wet towel wiping. When misting is performed via low-pressure nozzles, the cleaning efficiency is greatly enhanced, since cleaning conditions match closely those of vapor extraction and air migration of contaminants is minimized.

II. Surface Passivation (Optional). Surface passivation involves a mild acid/base treatment of the surface at low temperature (30−50 °C). Acid and base aqueous solutions are applied sequentially in a manner similar to the case of the gross cleanup. The objective is to mildly etch the surface and thus make contaminants, trapped inside microscopic capillaries of the surface, more accessible to the cleaning agent(s).

III. Exponential Cleaning. Exponential cleaning refers to the performance of a number of boil-outs, rinses, or mist-and-wipe applications, applied in a sequential fashion until a cleaning plateau has been achieved. Typically, the concentration of contaminants in the cleanup samples follows an exponential profile, which is consistent with an exponential dilution profile of the solute. Irrespective of the type of cleaning performed, a plateau is typically reached after 3−4 boil-outs, suggesting that cleaning is mass-transfer-limited, and that the composition of the cleaning solution must be enhanced with a lower surface tension constituent. To optimize the cleaning efficiency of a boil-out (minimize number of applications), the volume of the cleaning solvent must be kept to a minimum, so that the major load of cleaning is carried by vapor extraction.

IV. Microcleaning (Exterior Surfaces Only). Since rinsing is not as effective as boil-outs (vapor type of cleaning), low-pressure misting with the cleaning solution is the next most effective and efficient way of cleaning, when dealing with exterior surfaces. In such cases, the cleaning solution is applied via a low-pressure mist-generating spraying, and the surface is wiped dry using linear motions in one direction and without overlapping wiped areas. This procedure needs to be repeated 6−7 times to achieve maximum extraction efficiency. Again, when a minimum plateau is reached, the composition of the cleaning solution must be enhanced with the appropriate solvent constituent.

Case I: Cleaning at Pilot Scale. The following describes the cleaning strategy and results obtained following the pilot-scale manufacture of a potent anti-cancer substance (compound **C**) having an AWEL of 0.2 $\mu g/m^3$ (chemical strucure not shown). The operation involved a number of 300−500-gal reactors, a filter-dryer, a number of centrifugal pumps, and a nutsche filter. The cleanliness requirements were established for the entire reactor setup (∼2500-gal capacity, including pumps) to be <10 ppm of active substance in 50 L of cleaning solution; the limits for the filter-dryer and the nutsche filter were considered separately. Prior to obtaining

Table 1. **A pilot-plant case**

process equipment	cleanup agent(s)	cleanup description	activity (ppm /50 L)	air monitoring (μg/m^3)
		A. Lactose Placebo Test		
1 × 500 gal vessels + pump and piping	water, methanol	8 boil-outs	2	n/a[a]
		B. Actual Cleaning of Compound **C**		
five-reactor operation	acetone−water, methanol−water H_2SO_4−water, NaOH−water, HCl−water, water	2 × water rinses followed by 4 × boil-outs	activity from each equip set, after	processing areas,[b] <0.2
(reactors were divided in sets of two, including one pump plus associated piping)			1 boil-out: <1.5	personnel monitoring,[b] <0.1
(filter-dryer[c] and filter were cleaned separately)			2 boil-outs: <0.5	after the cleanup of spill,[b] <0.1
			4 boil-outs	
all equip sets combined	water	100 gal	2.0	

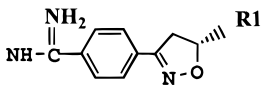
[a] Lactose was found to exist at background levels in blank samples. As a result, air-monitoring data were not conclusive. [b] Monitoring for 12 h. [c] The filter-dryer was cleaned to <0.2 ppm, which provided containment to a level of <0.1 μg/m^3.

the final sample, each of the reactors was sampled to ensure that individual activity levels did not exceed 2 ppm in 50 L of cleaning solution. The analytical (HPLC) limit of quantitation was set at 0.1 ppm, and no deactivating "kill" solution was available. During processing, an accidental spill of a significant amount of the (solid) drug substance took place inside a dryer room, adding to the complexity of the cleaning effort.

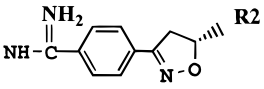
Due to the potency of the substance, great emphasis was placed on the design of the cleaning protocol. Approval of the cleaning rationale required consensus of the principal development engineer, chemist, and operations personnel, while solubility data and placebo testing results played significant roles in the design of an effective cleaning protocol. To assess the potential effectiveness of boil-outs, a cleanability test was performed prior to the design effort, using a concentrated solution of lactose as the placebo contaminant. The solution was circulated through each of the reactors and associated piping and pumps. Cleaning included the initial disposal of the reactor contents, application of two water rinses to remove excess lactose, followed by a sequence of four water and four water−methanol boil-outs. The final sample was obtained from a combined 100-gal rinse which was circulated through all reactors, pumps, and associated piping. When a minimum plateau at a concentration of 2 ppm was established, the cleaning effort was deemed complete. The choice of lactose as a placebo contaminant was thought to offer the benefits of a worst-case cleaning scenario without the health hazard implications; however, its natural presence in the air of the work environment (i.e., interfering with background air-monitoring) pointed out its limitations. In retrospect, the use of acetaminophen is now thought to be a more appropriate option. The results from the lactose testing and the actual cleaning are shown in Table 1.

The results shown in Table 1 provide us with a perspective on the level of cleanliness that can be achieved at a pilot scale, assuming that an appropriate choice of cleaning solution is made. The difference between the combined activity levels and those obtained from individual equipment sets indicates the existence of residual contamination in the pipe-fittings connecting the individual reactor sets. In such cases, the postcleaning disassembly and inspection of a select number of pipe-fittings in the setup need to be included in the requirements for cleaning validation.

CASE II: Cleaning at Laboratory Scale. Although the total quantity of material handled in a laboratory is limited, containment is not as easy, and the probability of exposure to potent substances during laboratory-scale preparations is appreciable. The following describes the cleaning efforts and investigation results obtained following an accidental laboratory spill involving a potent anti-thrombotic substance (Compound **A**). This spill, in addition to raising safety concerns about laboratory containment practices, presented the authors with an opportunity to explore the cleaning implications from such incidents, and accelerated efforts toward the development of a deactivation solution for two compounds belonging to the same class of anti-thrombotics (Compounds **A** and **B**). Both compounds were assigned an



Compound - A



Compound - B

AWEL of 2 μg/m^3, and the cleanliness criterion for contaminated surfaces was set at 10 μg/100cm^2. To measure cleanliness, contaminated surfaces were swab-tested, and results were reported as averages of multiple samplings obtained for various areas around the origin of the spill.

Exploring the Implications of a Laboratory Spill. The aforementioned spill incident involved the accidental breakage of a 6-L glass flask, containing compound **A** in the form of a slurry. During the incident, the contents of the flask splashed the interior of the hood space, where the flask was located, and a significant portion of the hood's exterior, including the floor tiles and the floor mat located in front of the hood.

Cleaning of the spill was conducted in four stages. In stage 1, the bulk of the contaminants was removed, and surfaces

Scheme 3. **Laboratory hood schematic**

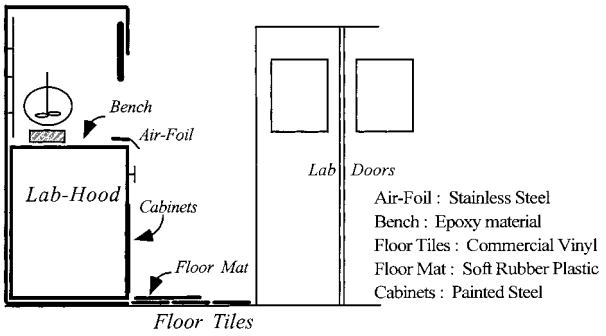


Table 2. **Gross cleaning results—compound A**

swab sample location	first stage of cleaning contamination level (μg/100 cm^2)
hood cabinets	800
lab floor by hood	400
lab floor by doors	125
outside lab corridor	≪10

Table 3. **Exponential/microcleaning results—compound A**

swab sample location	second stage of cleaning contamination level μg/100 cm^2)	third stage of cleaning contamination level μg/100 cm^2)
hood bench	700	≪10
floor tiles	90	10
floor mat	900−2800	70 (disposed of)
outside lab corridor	n/d	n/d
air-foil	100	≪10

were cleaned to a gross-clean level (100−1000 μg/100 cm^2); a number of swab samples were then taken to map the spread of contamination (see Scheme 3). As the results show in Table 2, no migration of the contaminant took place past the laboratory doors. The contamination profile generated from the swab samples was consistent with the nature of the spill. In stage 2, all disposable items inside the hood were collected for incineration, and the hood's air-foil was removed to facilitate further cleaning. This time, cleaning was performed in a more systematic manner (exponential cleaning). Surfaces were wiped 3−4 times using paper towels moistened with a methanol−water solution; each time, a clean paper towel was used until surfaces appeared to be visually clean. Swab samples taken from various areas inside and around the hood indicated that the cleaning effort had plateaued at a level significantly higher than that of the desired action limit of 10 μg/m^3 (see Table 3). Next, the "microcleaning" approach was implemented. Surfaces were misted with a methanol−water solution and wiped dry using new paper towels each time. This time (see Table 3, stage 3), significant improvement in cleaning was achieved, with the only exception being the floor mat, which was discarded by incineration. To minimize the risk of exposure during the disposal of the floor-mat, its surface was sprayed with a vinyl coating to entrap any contaminants. Subsequent swab testing of the floor mat produced negative results, indicating that the vinyl coating provided an effective barrier against any potential release of contaminants. Such an approach, although it offers temporary containment, should be applied to disposable surfaces only.

Table 4. **Recovery Study:[a] surfaces spiked at 10 μg/100 cm^2 level—compound A**

surface description	methanol (%)	ACN/water (%)
stainless steel (smooth, not polished)	70	70
epoxy bench top	80	70
acid brick	100	75
Teflon	75	65
glass	65	40
vinyl floor tile	<35	0

[a] Recovery of 10 μg/100 cm^2 measured by HPLC (height of peak from baseline ± 5).

While swab test results met the cleanliness requirements set forth prior to cleaning, knowledge of the percent recovery achieved by the cleaning procedures employed (discussed later in the paper) was deemed essential in assessing the overall efficiency of the cleaning effort. In obtaining such data, a spike and recovery study was conducted. The study involved materials similar to those exposed by the spill. Also, two different swabbing solvents were used to evaluate their impact on recovery: methanol and a 1:1 mixture of acetonitrile−water.

The results from the recovery study are shown in Table 4. With the exception of the vinyl tiles, all surfaces involved produced relatively high recovery, with the highest being those extracted with methanol. Thus, methanol was chosen as the preferred solvent for all subsequent swab testing. The most interesting result observed in this study was that involving the acid-brick, which produced 100% recovery. This was attributed to the baking of bricks at high enough temperatures, which reduces their surface porosity. Unexpectedly, the glass surface produced poorer recovery, despite its apparent smooth finish. Also, in the case of the floor mat, the use of acetonitrile−water appeared to have a significantly negative effect on recovery: 0% recovery was achieved. This was attributed to the acetonitrile's solvating strength causing contaminants to become entrapped in the matrix of the mat's surface. Overall, the recovery data suggest that a 50−70% recovery should be considered the default value for swab analyses purposes when dealing with laboratory and manufacturing environment surfaces.

Addressing the Analytical Issues. *I. The Assay Method.* An action limit of 10 μg/100 cm^2 can present a detection limit challenge if quantitation of concentrations near 0.1 μg/mL is required. Techniques such as UV spectroscopy are not sensitive enough to resolve analytes from interferences caused by various residual extractables. On the other hand, HPLC has been found to provide both good separation of the analyte from extractables and good sensitivity.[8] In this study, initial analyses were performed using a Hewlett-Packard HPLC system equipped with a Zorbax SB-C8-75-

(8) Shea, J. A.; et al. Validation of Cleaning Procedures for High Potent Drugs. Pharm. Dev. Technol. **1996**, *1* (1), 69−75.

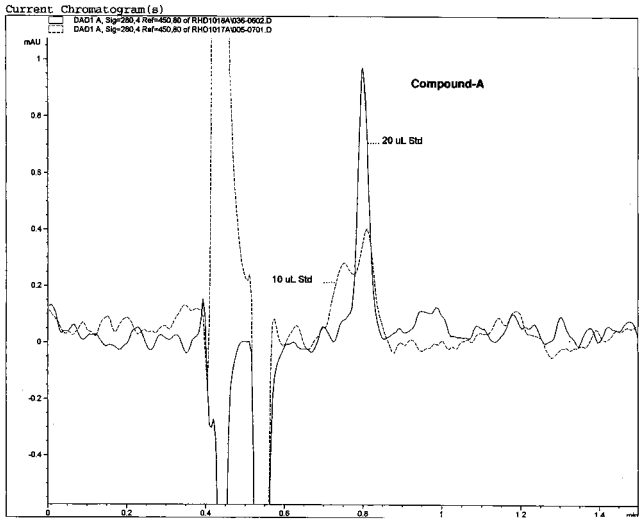


Figure 2. **Effect of matching the sample solvent to the mobile-phase solvent.**

mm × 4.6-mm column and a UV detector set at 280 nm. Other settings included mobile phase of 1:4 ACN−water (with 0.1% trifluoroacetic acid), pump flow rate set at 1 mL/min, and column temperature set at 40 °C. Later, during the development of a deactivating solution, a longer column (250 mm × 4.6 mm) was used to ensure better resolution of the degradation products.

In achieving the necessary separation and sensitivity, several techniques were utilized. First, a wavelength of 280 nm was selected to be selective and sensitive to both compounds, relative to solvents and other extractables. To reduce peak distortion (peak splitting), the solvent ratio of the extraction solvent (1:1 ACN−water) was diluted with more water to closely match that of the mobile phase (1:4), while the injection volume was increased to 25 μL to compensate for the dilution; typically, analyses using a 4.6-mm-i.d. column require 5−10-μL injection volumes. As a result of the simultaneous increase of the injection volume and dilution of the sample, the absolute sensitivity was enhanced. Figure 2 depicts a comparison between the peak shape obtained from a 10-μL injection of a 0.5 μg/mL sample of compound **A** prepared in 1:1 ACN−water to the peak shape obtained from a 20-μL injection of a 0.25 μg/mL sample prepared in a solvent matching the HPLC mobile phase (1:4 ACN−water). The sample prepared in 1:1 ACN−water ("stronger" solvent) is distorted and split, while the standard prepared in 1:1 ACN−water is taller and easier to quantify.

II. Swabbing Testing. One of the most important parameters in swab analysis is percent recovery, that is, the fraction of the contaminant (analyte) that can be recovered from a surface and accounted for by assaying. Recovery depends on a surface's finish characteristics and the contaminant's affinity to become adsorbed onto that surface. Recovery estimates are obtained by conducting spike and recovery studies using representative surfaces. Since the action limit in this investigation was at 10 μg/100 cm^2, a number of 2-in. × 2-in. plates of various construction materials were spiked with 10 μg of compound **A** in methanol. The spiked plates were allowed to dry and then swabbed, using 2-in. × 2-in. cotton gauzes, moistened with an extracting solvent; the gauzes were folded in half and handled with forceps to avoid exposure. Following the swabbing process, the gauzes were extracted into HPLC-grade water and further diluted to a 50-mL volume with a sufficient amount of acetonitrile to match the HPLC mobile phase. Sufficiently large injection volumes were then made to achieve the desired sensitivity. Stardard solutions were prepared in 1:4 acetonitrile−water at concentrations ranging between 0.05 and 0.24 μg/mL.

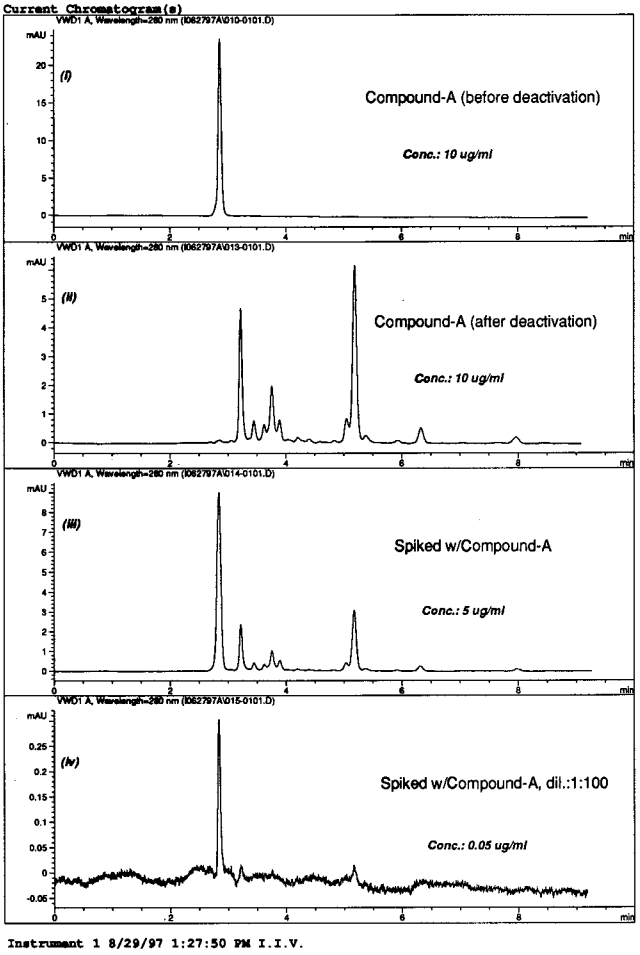
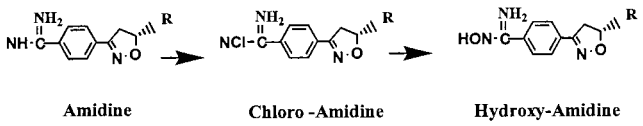


Figure 3. **Deactivation of compound A.**

Scheme 4. **First stage of amidine deactivation**



Developing a "Kill" Solution for Compounds A and B. Efforts toward finding a deactivating solution for this class of anti-thrombotic compounds led to the discovery that amidines similar to compounds **A** and **B**, in the presence of sodium hydroxide and sodium hypochlorite (bleach), undergo two stages of chemical reactions. In the first stage, the amidine converts to its hydroxyamidine derivative via the formation of a chloroamidine intermediate (Scheme 4). In the second stage, at increased concentrations of NaOH and bleach, fragmentation of the hydroxyamidine to lower molecular weight species takes place. Although the mechanism for the second-stage reactions is not well understood,

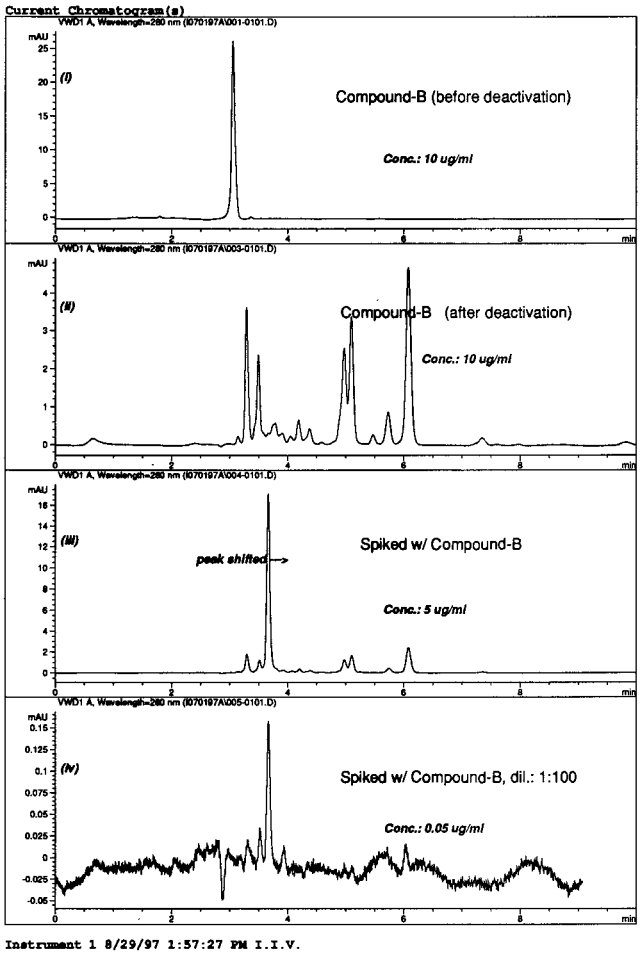


Figure 4. **Deactivation of compound B.**

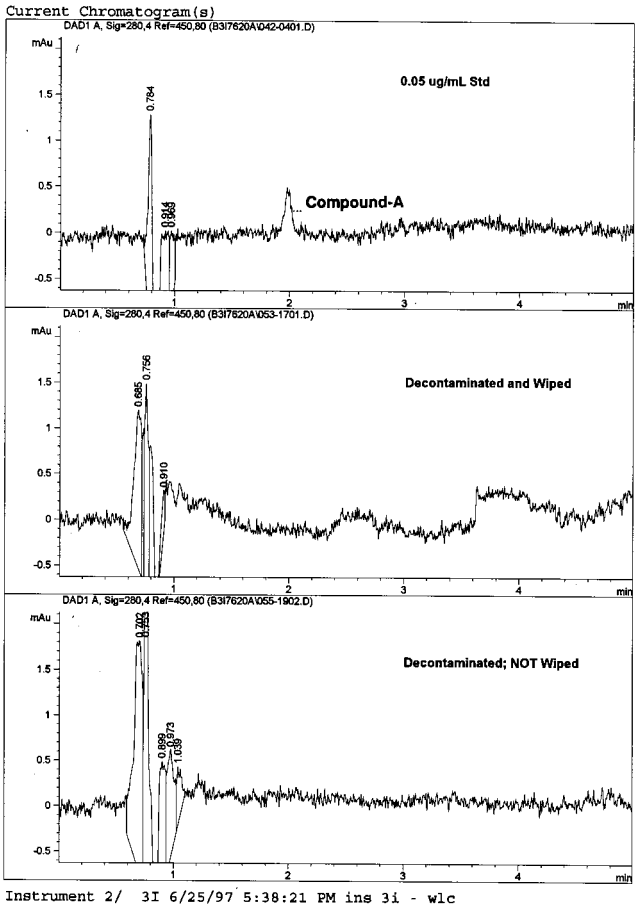


Figure 5. **Swab testing applied on stainless steel plates.**

the fragmentation was attributed to the oxidative power of bleach attacking the two chiral centers of the molecules (the second chiral center, in functional group "R", is not shown). Analysis by mass spectroscopy confirmed the absence of the parent amidine species, with the larger molecular fragments having a mass about half that of the parent species. Testing isolated residues of the degradation byproducts for "platelet aggregation" resulted in no activity.[9] These results confirmed the effectiveness of the NaOH/NaOCl mixture as a "kill" solution for compounds **A** and **B**.

Next, the formulation of a decontamination procedure that would meet both the laboratory and manufacturing needs was developed. The procedure involved the use of two solutions: (i) a deactivating solution consisting of 25 vol % methanol, 22.5 vol % water, 0.2 wt % NH_4Ac, 50 vol % NaOCl (5−6%), and 2.5% NaOH (50%) and (ii) a neutralizing solution consisting of 30 vol % methanol, 60 vol % water, and 10 vol % acetic acid. The concentration and composition of the deactivating solution ensured rapid dissolution and deactivation of the respective compounds. By following the decomposition profile via HPLC, it was estimated that 100 L of that solution would effectively deactivate 500 g of either substance within 5 min. To ensure solubility of the degradation products and deactivation of any residual NaOCl, the neutralizing solution was used. Addition of 0.2 wt % of ammonium acetate into the deactivating solution compensated for the instability of the two compounds in methanolic solutions. Figures 3(ii) and 4(ii) show the results from the deactivation of compounds **A** and **B**. Fragmentation of the respective molecules produced a number of degradation products. Figures 3(iii) and 4(iii) show the chromatograms after spiking the samples containing the degradation products with an equivalent amount of the parent compounds. In the case of compound **B**, a slight shift in retention was noted, attributed to basic pH. Both compounds could be detected at concentrations as low as 0.05 μg/mL, 50% of the desired action limit.

The decontamination procedure involved spraying a surface with the deactivating solution and wiping the surface dry, after allowing 1−2 min to pass. To ensure the complete decontamination of a surface, the sequence was repeated at least 4 times, using a clean drying towel each time. Prior to obtaining a swab sample, the surface was sprayed with the neutralizing solution and dried once more.

This procedure was tested on compound **A**, by performing a spike study on 100 cm^2 stainless steel plates. To ensure the effectiveness of the method on a worst-case scenario, the finish of the plates was mildly coarsened. The recovery of compound **A**, which was first established by swabbing a plate spiked with 10 μg of compound **A**, was calculated to be in the rage of 50−60%. As a result, the swab sample concentration requirement was lowered from 0.1 to 0.05 μg/

(9) Mousa, S. A.; et al. A Novel Platelet GPIIb/IIIa Receptor Antagonist. *Cardiology* **1993**, *83*, 374−382.

mL. Next the spiked plates were sprayed with the deactivating and neutralizing solutions. One of the plates already sprayed with the deactivating solution was allowed to air-dry, instead of wiping it dry with paper. All plates were swab tested against a 0.05 μg/mL standard solution (see Figure 5(i)). The HPLC chromatogram of the standard solution appears to be easily measurable, well above the baseline noise. The chromatogram of the air-dried plate (see Figure 5(iii)) shows no residue of compound **A**, which implies that its removal was due to deactivation and not due to adsorption on the paper wipes. Overall, this swab study demonstrated the effectiveness of the decontamination procedure in cleaning compound **A** to very low levels.

Acknowledgment

We would like to thank our colleagues, past and present, for offering their assistance and sharing their experiences on the handling of potent compounds. Especially, I.I.V. would like to acknowledge the contributions of Dr. L. H. Zhang and Dr. H. Li in identifying an effective deactivating solution for the anti-thrombotic compounds A and B. Last, I.I.V. would like to thank Mrs. Freida Fisher-Tyler and Mr. Craig Wood for their constructive feedback on industrial hygiene issues.

Received for review January 1, 1998.

OP980181D