Cleaning verification assays for highly potent compounds by high performance liquid chromatography mass spectrometry: Strategy, validation, and long-term performance

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Abstract

A cleaning-verification assay was validated for a highly potent family of compounds utilizing a swab-sampling procedure and high performance liquid chromatography mass spectrometry (LC-MS) for separation and detection of the analytes. Due to the high potency of the compound, the LC-MS method was validated at a level of 50 ng/25 cm^2 and 50 ng/100 cm^2 (which equates to 10 ng/ml after extraction in 5 ml of sample solvent, and 3 ng/ml after correction for sampling losses). This validation exercise included recovery estimates from all drug product contact surfaces within the clinical trial manufacturing equipment, namely, stainless steel, anodized aluminum, Rilsan® coated aluminum, bronze, polyvinylchloride, and Oilon®. The limit of detection for the LC-MS method was determined to be less than 0.5 ng/ml, or less than 0.1 ng/cm^2, of the analyte. This method does not employ an internal standard. Long-term performance of the validated method is also reported. The precision on replicate injections of the standard prepared in the range of 3–6 ng/ml was typically better than 8.0% relative standard deviation (R.S.D.) over the course of 1 year, which resulted from 10 cleaning-verification submissions. Those results were consistent with the data obtained during method validation.
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1. Introduction

Cleaning verification can be a challenging analytical problem in the pharmaceutical industry when, for example, a compound is considered to be extremely potent, possesses a poor chromophore, or the combination of the two. In the pharmaceutical manufacturing/packaging process, it is important to ensure the production equipment is properly cleaned in order to avoid cross-contamination of drug products [1]. The removal of drug residues is typically conducted by a series of cleaning procedures that can include acidic, basic, and detergent based cleaners. A cleaning verification program confirms the effectiveness of those cleaning procedures during Phase I and Phase II clinical trials; during this time, analytical measurements provide a high degree of assurance that the target drug residue is below the safety acceptance criteria. Typically, the analytical method employed involves high performance liquid chromatography (HPLC) with

ultra-violet (UV) detection [2–4]. The acceptance criteria are established to ensure human safety and are based on toxicological and pharmacological data, dose strengths, equipment surface area, manufacturing batch size, number of dosage units per batch, smallest strength manufactured, and product contact surface area [5,6]. In general, a lower cleaning acceptance limit is required for a smaller dose strength, as it is assumed to be more potent. In order to produce an adequate margin of safety, one approach is to employ a risk factor of 1/1000 to the no-effect or low-effect dose levels used in the clinical trial [7]. In cases where the therapeutic dose is projected to be in the low micrograms per day or per capsule range, the cleaning-verification acceptance limit is driven into the nanogram regime. For example, a dose of 10 μg API (active pharmaceutical ingredient)/capsule might require that the clinical trial manufacturing or clinical trial packaging equipment be cleaned to a level below 50 ng/25 cm^2 or 50 ng/100 cm^2, respectively. In this work, a 10 cm × 10 cm area is swabbed for CT packaging as opposed to a 5 cm × 5 cm area for CT manufacturing, due to equipment and lot size differences. Different swabbing areas were devised to assist the analytical chemist with sensitivity problems on compounds with

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low acceptance limits. A factor of four improvements in sensitivity was theoretically gained (does not account for lower recovery typically observed on larger surface area) by increasing the surface area by the same factor. With a direct-sampling cleaning-verification strategy (i.e. swabbing), this acceptance limit of 50 ng/swab will result in analytical samples that are approximately 10 ng/ml with a 5 ml dilution in sample solvent. Although it is not impossible to reach this limit with conventional HPLC-UV when the compound of interest has a high molar absorptivity, it can be difficult for most compounds.

When dealing with low-level cleaning verification assays, several factors contribute to the complexity of the analysis. When considering that the allowable API residue may be 2 ng/cm^2 or less, the first difficulty arises during sampling of the surfaces with swabs. Such a low limit makes the sample extraction from a surface challenging during the swab sampling process, due to some level of affinity of the analyte to the surface. The small amount of analyte absorbed onto a surface, or unextracted from the swab, may account for only a small fraction of the total analyte when at a relatively high acceptance limit. However, the same absolute amount of loss becomes a significant fraction of the total analyte when the acceptance limit is low. As a result, lower recovery from a surface may be expected as the dose is reduced and is dependent upon the finished characteristics (i.e. rough surface versus smooth surface, or polymeric surface versus metal surface) of the surface and its affinity to an analyte. Therefore, the previously cited limit of 50 ng/25 cm^2 or 50 ng/100 cm^2 could result in an analytical sample that is 2–5 ng/ml when corrected for the above-mentioned recovery challenges. The combination of low acceptance limits and potentially low recoveries can present a significant analytical challenge. At such a low level, the detection limit may not be easily achieved with HPLC-UV. Other detection techniques used in conjunction with HPLC, such as fluorescence and electrochemical detection, are effective and afford excellent sensitivity, but are compound specific. As a result, the advantages associated with these techniques are limited to a smaller subset of compounds. Mass spectrometric (MS) detection, on the other hand, is applicable for many classes of pharmaceutically relevant compounds.

There have been several examples in the literature where extremely low-level cleaning verification assays have been validated with HPLC-UV. For example, Shea et al. developed a cleaning verification assay utilizing HPLC-UV for the determination of Losoxantrone, which is a cytotoxic compound for breast cancer [8]. In addition to illustrating a step-by-step approach for developing a cleaning verification assay, they demonstrated that the HPLC-UV method had a detection limit of 2 ng/ml. In another example, a HPLC-UV assay was developed for the compound Bisnafide, which is also a cytotoxic compound [9]. For this assay, the method was sensitive to 4 ng/ml of Bisnafide. An interesting approach presented by Raghavan and Mulligan involved the use of atomic absorption (AA) spectroscopy. The determination of cisplatin in cleaning validation rinse solutions was performed with a very sensitive AA method [10]. In this work, cisplatin was first derivatized with diethyldithiocarbamic acid to yield a platinum complex

that was subsequently detected by AA. The sensitivity of this method was reported to be 0.5 ng/ml of cisplatin. Valvis and Champion presented a strategy for cleaning and decontamination of potent compounds by establishing meaningful criteria for acceptance criteria as a foundation for developing a sound analytical method [7]. They reported an analytical method that was capable of detecting two unspecified compounds at 0.05 μg/ml. In a separate work, an HPLC method was validated for fentanyl, alfentanil, and sufentanil in swab samples [11]. Efficient chromatography with moderate run times (<12 min) and high sensitivity were achieved for all compounds. Detection limits were reported to be from 2 to 10 ng/ml for this class of compounds by utilizing wavelengths that corresponded to the maximum absorbance of each compound.

On the use of LC-MS for cleaning verification, Forsyth and Van Nostrand presented that method development times and sample injections are generally shorter [12] In addition, the sensitivity can be the same as HPLC-UV, or better. However, they consider the LC-MS method to be less rugged and more costly than conventional HPLC with UV detection. Recently, Kolodsick et al. reported an excellent application of LC-MS/MS (utilizing a triple quadrupole mass analyzer) for enhanced sensitivity and specificity of drug residues for cleaning validation in manufacturing equipment [13]. The work by Kolodsick advocated the use of internal standards to correct for ion suppression effects. Two alternatives were illustrated: (1) isotopically labeled analytes and (2) structural analogs. Data were presented on linearity and precision improvements achieved through the use of internal standards. Sensitivity requirements for method validation were satisfied with a low level solution at 10 ng/ml, which resulted in detection limits in the range of 0.02–0.2 ng/ml for all compounds investigated.

For pharmaceutical compounds, LC-MS has found extremely wide acceptance due to the low-level detection that can be achieved, in addition to the selectivity and specificity that are attained by using HPLC in conjunction with MS detection. The goal of this work is to demonstrate that LC-MS can be the preferred methodology, and a universal technique, for low-dose cleaning-verification applications due to its specificity, low detection limit, and long-term consistent performance. The work presented here investigates method performance over an extended duration without the use of internal standards and will also demonstrate that a generic method can easily be developed and applied to all the compounds in a particular class due to the specificity afforded by LC-MS.

2. Experimental

2.1. Chemicals and materials

HPLC grade of methanol, ammonium acetate, and glacial acetic acid were obtained from Fisher Scientific (Pittsburgh, PA, USA). Water was deionized and filtered through a Millipore Milli-Q® water purification system (Bedford, Massachusetts). Alpha swabs (Texwipe® 714A) were from Texwipe Co. (Upper Saddle River, New Jersey). Falcon® 15-ml polypropylene tubes were from BD Labware (Cockysville, Maryland).

Test surfaces were constructed in house with dimensions of 5 cm × 5 cm, or 10 cm × 10 cm. Materials evaluated were representative of product contact surfaces in the clinical trial manufacturing and packaging areas and include stainless steel, anodized aluminum, Rilsan® coated aluminum, bronze, polyvinylchloride, and Oilon®.

2.2. Equipment

The experiments were performed on Agilent 1100 series HPLC system and ion trap mass spectrometer (SL model), equipped with electrospray ionization, from Agilent Technologies (Palo Alto, CA, USA). Reference Table 1 for operating conditions of both the HPLC and mass spectrometer utilized in this study.

2.3. Standard preparation

A stock standard was prepared by weighing approximately 5–10 mg of standard material into a 500 ml volumetric flask, and dissolving it in methanol. An intermediate spiking standard was prepared by making a dilution in methanol from the stock standard. Methanol is a convenient spiking solvent since it dries rapidly which allows development and validation exercises to proceed without extensive drying times. A final ana-

Table 1
LC-MS method summary

Chromatographic conditions	
Column	Zorbax RX C18, 150 mm × 4.6 mm, 5 μm
Column temperature	30 °C
Mobile phase	80% Methanol/20% pH 4.5 ammonium acetate buffer
Flow rate	0.4 ml/min
Injection volume	100 μl
Mass spectrometer and data acquisition parameters	
Dry temperature	350 °C
Nebulizer pressure	50 psi
Drying gas	10 l/min
Data acquisition	30 ms, 10 spectra average
Optimized voltages for ion trap	
Electrospray	3500 V
Capillary exit	−125 V
Trap drive	49 V
Data acquisition for ion trap parent ion	
Maximum accumulation time	30 ms
Average spectra	10
Scan mode	400–440 m/z
Data acquisition for ion trap fragment ion (MS/MS)	
Maximum accumulation time	200 ms
Average spectra	4
Scan mode	310–330 m/z
Optimized voltages for single quadrupole	
Electrospray	3700 V
Fragmentor	90 V
Data acquisition for single quadrupole	
Scan mode	SIM

lytical working standard was typically prepared in the range of 2.5–10.0 ng/ml by making a dilution in 1:1 methanol:ammonium acetate buffer (pH 4.5–5.1), from the intermediate standard. Other standard concentrations used for development purposes will be specified in the text. The 10 mM ammonium acetate buffer was prepared by adding 0.77 g of ammonium acetate to 1 l of water and pH adjusted with acetic acid.

2.4. Swab sample preparation

Spiked surfaces were prepared by adding 50–100 μl of the spiking standard onto the surface, and allowed to dry prior to swabbing. The amount of spiking standard added to the surface was at a level equivalent to the safety limit, which had been previously established as 50 ng/surface. A swab sample was prepared by wiping, 10 times horizontally on one side of the swab, flipping the swab and then wiping 10 times vertically on the other side of the swab. A pre-rinsed methanol-soaked swab was utilized to swab on the selected surfaces. Methanol was chosen because of the solubility of the compounds in methanol, and because it is an acceptable solvent to use in the clinical trial manufacturing area. Each swab sample was then placed in a Falcon® 15-ml polypropylene tube. The analyte was extracted from the swab by adding 5.0 ml of 1:1 methanol:ammonium acetate buffer and hand shaking for approximately 1 min. An aliquot was then taken for injection onto the HPLC-MS system.

3. Results and discussion

3.1. Strategy for method development and validation

Cleaning verification includes sampling (e.g. swabbing) manufacturing/packaging equipment at multiple pre-defined product contact surfaces, which are typically described with rationale in a clinical trial master study plan. Cleaning verification, as opposed to cleaning validation, requires that swabs are submitted for multiple surfaces after each manufacture to ensure that equipment is suitably clean for the subsequent manufacture. As a result, the analytical recovery of analyte from each individual representative surface type should be considered. In this work, the surface recovery was used to establish the assay pass/fail limit (APFL) in ng/ml, which is defined in the following equation:

$$APFL = \frac{\text{Acceptance limit per swab (ng)}}{\text{Swab extraction volume (ml)}}$$
$$\times \frac{\text{Surface recovery (\%)}}{100\%}$$

In a limit test where the worse-case recovery is utilized, the APFL simply defines the concentration of the standard preparation. Preparing a standard at the limit, as opposed to a mathematical correction, is preferred because it serves as a sensitivity check for the analysis. For a limit of 0.5 ng/cm² and a sampling area of 100 cm², the limit per swab becomes 50 ng. The best-case scenario, assuming 100% recovery and using 5 ml extraction solvent, APFL becomes 10 ng/ml (the concentration of the standard). From this example, it is apparent that the limit of

quantitation must be less than, or equal to, the APFL. Thus, the quantitation limit becomes the most critical validation parameter when the safety acceptance level is low.

This method was validated as a limit test. The work by Kolodsick [13] was very meticulous in that an internal standard was utilized for quantitation between 10 and 100 ng/ml. The authors agree that the use of internal standards provides the most precise and accurate means of performing quantitative LC-MS. However, the method validation strategy outlined here employed a limit test in order to expedite method development and validation during early clinical trials while still maintaining patient safety. During the method validation, the worst-case recovery was utilized to establish the APFL. For example, if a recovery of 50% was obtained for cast iron and a recovery of 95% was obtained for stainless steel, a recovery of 50% was utilized as the APFL for all surfaces when the swab assay was executed. Although conservative, this approach ensured that equipment is never passed as "clean" that should have failed the cleaning verification. In addition, there is an expectation that a safety margin of 1000 is built into the calculated limit. For the low-dose-case, a 50% recovery versus a 70% recovery would be the difference between 5 and 7 ng/ml, which equates to a safety margin of 1000 and 1400, respectively. By utilizing the worst-case recovery, the safety margin of 1000 is always maintained. In addition, this strategy simplifies the calculating, reporting, and verification of data. On the other hand, there is a slight risk that equipment that should have marginally passed cleaning verification could inadvertently be failed. With this approach, the equipment is either clean (<50 ng/swab), or it fails (≥50 ng/swab). If the clinical trial manufacturing area requests a semi-quantitative estimate (e.g. how "dirty" was the equipment?), it can be provided with reasonable confidence. Regardless, the equipment that failed is cleaned, sampled again, and swabs are re-submitted to the labs. Therefore, no quantitative numerical data is provided to the clinical trial area on a routine basis.

Throughout development and validation, precision was observed to be better than 10% relative standard deviation (R.S.D.) indicating that the method is operating below the limit of quantitation. This variance results in a deviation of only ±1 ng/ml when the analyte is at a concentration of 10 ng/ml. Upon method execution, multiple standards (same concentration) are determined throughout the analysis. The standard that produces the lowest peak area is used for comparison as the APFL. This approach is more conservative than using the average, but less stringent to develop and validate than a quantitative method. Again, the safety margin of 1000 is maintained at all times.

It is worth pointing out that the method utilizes conventional dimensions for HPLC columns and does not require a smaller column or micro-bore plumbed HPLC in order to improve sensitivity. Although the detection limit could be further improved by making these changes, the purpose of the work is to establish a generic method that meets the sensitivity requirements for all of the new chemical entities (NCE) investigated, and can be easily executed, or transferred to a quality control lab, without the need for modifying standard HPLC equipment.

3.2. Method development

The compounds presented in this paper are new chemical entities at Eli Lilly and Company selected for multiple indications and will be represented as NCE 1–4 throughout this discussion. The doses tested in this study are 10–100 μg/capsule. Based upon dose and typical lot sizes, cleaning verification safety limits have been established from 0.5 ng/cm^2 (clinical trial packaging) to 2 ng/cm^2 (clinical trial manufacturing) to ensure that there is no carryover of these potent compounds to the next clinical trial lot where the dose may be much higher.

All NEC's possess conjugated double bonds and exhibit good absorbance in the UV. Therefore, a preliminary evaluation was conducted using a conventional HPLC-UV system. However, the required detection limit at this low level was difficult to meet, even using a somewhat unorthodox injection volume of up to 1800 μl.

Of particular structural note in relation to mass spectral detection is that all NCE's are carboxylic acids, and all compounds will respond in positive and negative ionization modes for mass spectral detection. Their molecular weight ranges from 400 to 500 g/mol.

The chromatographic and MS conditions were developed quickly to achieve adequate selectivity and sensitivity. The parameters evaluated included flow rate, pH, ionization mode, spray chamber parameters, capillary voltage, capillary exit voltage, and trap drive voltage. No attempt was made to optimize the extraction volume, since the detection limit was met. The injection volume was 100 μl for all experiments. The maximum injection volume should be utilized in order to improve sensitivity without sacrificing chromatographic peak shape. Fig. 1 shows a chromatogram using the LC-MS method developed in this work. The concentration, in this case, ranged from 9 ng/ml (NCE 1) to 18 ng/ml (NCE 4). This figure represents an overlay of three injections. The results immediately demonstrated that adequate detection and specificity were attainable with minimal method development for the entire class of compounds investigated. Table 1 summarizes the LC-MS method conditions that
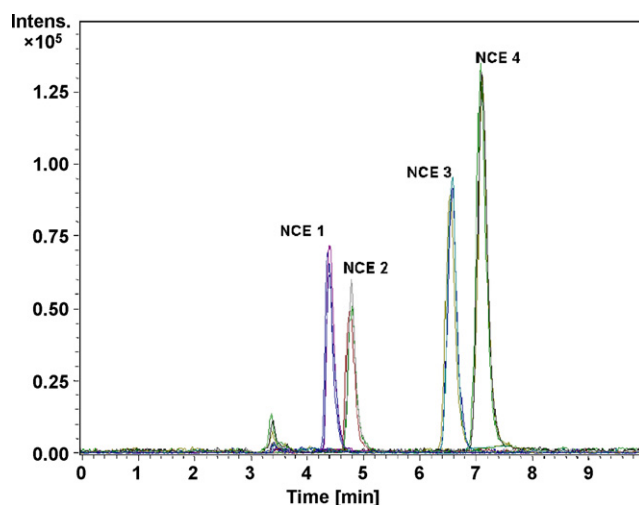

Fig. 1. Results of using the generic method described in Table 1. Each chromatographic peak represents three replicates of the NCE's.
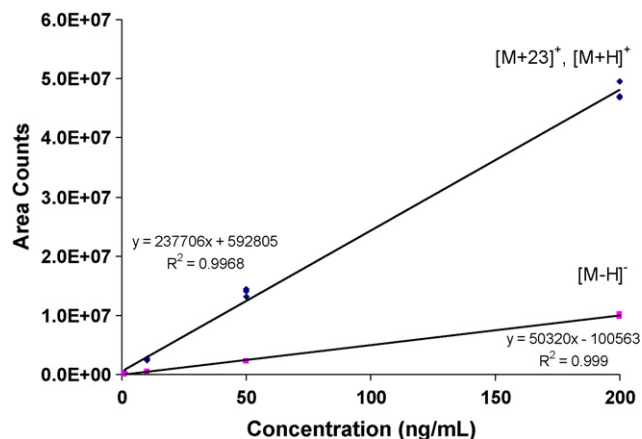
Fig. 2. Assessment of positive-ion electrospray vs. negative ion electrospray, for NCE 1.

were eventually validated and utilized for the cleaning verification assays.

Using the chromatographic conditions outlined in Table 1, both positive and negative electrospray ionizations modes were evaluated. Fig. 2 illustrates the linearity obtained from both ionization modes, using NCE 1 as an example. For positive mode, 0.05% trifluoracetic was used to lower the pH in order to promote protonation of the molecules. The difference in pH also explains the slight shift in retention time that is observed between Fig. 3a and b. For the positive ionization mode, there are two dominant ions present at approximately the same level; these ions result from protonation, H^+ (M + 1), and a sodium adduct, Na^+ (M + 23), respectively. The linear curve for positive ion electrospray in Fig. 2 utilized both ions of $[M + H]^+$ and $[M + Na]^+$. Although the two combined positive ions showed much higher response factor than the negative ion, the estimated detection
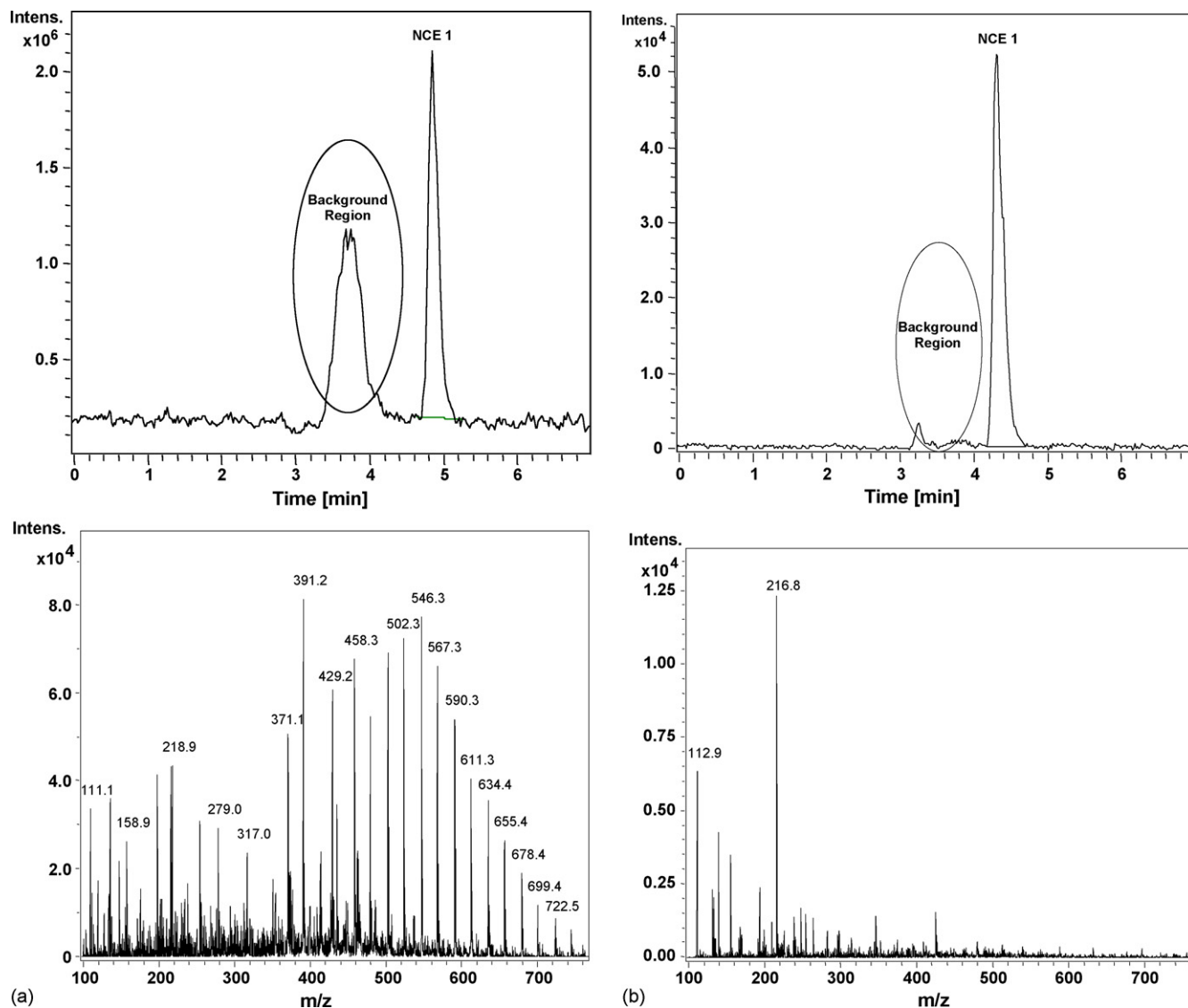

Fig. 3. Reduced background with negative ion electrospray. (a) Positive ion-electrospray of 10 ng NCE 1/ml. NCE 1 retention time equals 4.9 min. Substantial background peak between 3 and 4 min sometimes elutes later in chromatogram. Mass spectrum shows a complex polymer related background. (b) Negative ion-electrospray of 10 ng NCE 1/ml. NCE 1 retention time equals 4.5 min. Substantial background peak between 3 and 4 min is eliminated in this mode. Mass spectrum for the region between 3 and 4 min is essentially background ions.

Table 2
Recovery and precision data for NCE 1 and NCE 2 from all product contact surfaces

Surface	NCE 1 ($n = 6$)		NCE 2 ($n = 3$)	
	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)
None (spiked swab)	101	3.0	NE[a]	NE[a]
Stainless steel, 5 cm × 5 cm	82	5.3	NE[b]	NE[b]
Stainless steel, 10 cm × 10 cm	59	17.6	41	16.0
Anodized aluminum, 5 cm × 5 cm	66	9.0	70	3.5
Rilsan® coated aluminum, 5 cm × 5 cm	99	1.4	66	3.8
Bronze, 5 cm × 5 cm	31	11.5	48	2.2
Polyvinyl chloride (PVC), 5 cm × 5 cm	91	4.2	73	5.6
Oilon®, 10 cm × 10 cm	79	4.5	NE[c]	NE[c]

[a] Not evaluated: validation protocol was revised to only evaluate the overall recoveries which is the combination of swab recovery and surface recovery.
[b] Not evaluated: a worse-case recovery on the 10 cm × 10 cm stainless steel surface was utilized to estimate the recovery for NCE 2.
[c] Not evaluated: it was not a product contact surface for the manufacture of this compound.

limit of the positive ions are approximately three times worse than the negative ion due to higher baseline noise. Thus, the detection limit for each individual positive ion (i.e. either the $[M + H]^+$ or the $[M + Na]^+$) would be approximately six times worse than the negative ion $[M − H]^−$.

During the method development, late eluting broad peaks sometimes appeared during subsequent injections. The data in Fig. 3a were collected in positive ion mode using 0.05% trifluoroacetic acid as the modifier, in order to promote protonation of the molecule. The oval on the chromatogram illustrates the elution region for the background peak. The mass spectrum demonstrates that this chromatographic peak is a distribution of multiple masses. These peaks could be a result of slight solubilization of the Texwipe® swabs (polyester tip) in the organic solvent. This peak was apparent in blank swabs and swabs that had been used for swabbing surfaces; however, it was not there when running the standard (see Fig. 4). Regardless of the origin, these polymer peaks ionize extremely well in positive electrospray, but ionize very weakly in negative electrospray mode, see Fig. 3b. In Fig. 3b, the oval again represents where this peak would elute in the chromatogram. The mass spectrum associated with this elution region shows a fairly clean background spectrum particularly in the molecular weight (m/z) region of interest between 400 and 500 m/z.

Based on the above data, negative-ion electrospray was selected as it provided lower detection limits and a reduction in chemical background noise. Full validation was performed for NCE 1 and NCE 2. The validation for NCE 3 and NCE 4 was not completed due to the fact that the projects were discontinued. However, these methods could be validated quickly with the operating conditions outlined in Table 1, since the selectivity and sensitivity were demonstrated in Fig. 1.

3.3. Method validation

The method was validated for specificity, limit of detection, precision, surface recovery, and the solution stability of standards and of the extracted swab sample solutions. In addition, the stability of the swab samples prior to extraction was evaluated in order to determine the allowable time interval between swabbing clinical trial equipment and extraction of the analyte from the swab with sample solvent. Twenty-four hour stability was determined for swab samples before and after extraction, and for the standard solutions. The recovery and precision data are summarized in Table 2. The significantly lower recovery from 10 cm × 10 cm than 5 cm × 5 cm stainless steel for NCE 1 was noticed. The surface area of 10 cm × 10 cm is four times of that of 5 cm × 5 cm, making the recovery of a trace level analyte more difficult, even though 20 horizontal and 20 vertical swipes were used for swabbing on the larger surface. For NCE 2, it was decided to only evaluate a 10 cm × 10 cm surface as a worse-case recovery. The recovery from bronze surface was somewhat lower for both NCE's, compared to the other 5 cm × 5 cm surfaces. This surface is rougher than the other surfaces, which may attribute to lower analytical recovery. After incorporation of the sample dilution factor and the surface recovery, the final concentration for the APFL standard became approximately 3 ng/ml for the worst case. That is, the lowest recovery was 31% for NCE 1, and 41% for NCE 2, resulting in an APFL of 3.1 ng/ml (bronze surface) and 4.1 ng/ml (stainless steel surface) for NCE 1 and NCE 2, respectively. The limit of detection for both methods was calculated to be less than 0.5 ng/ml. This detection limit is
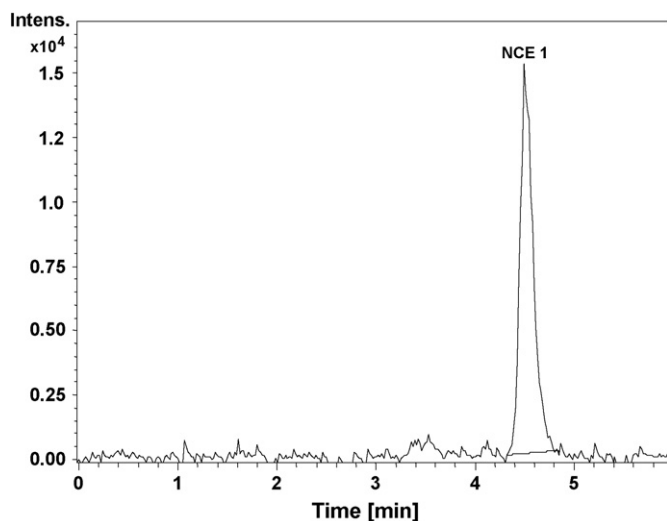

Fig. 4. Chromatogram for NCE 1 at 3.0 ng/ml. This concentration is utilized as the APFL and system suitability check after recovery correction of 30%.

Table 3
Long-term performance for NCE 1 demonstrated over the course of 1 year for multiple cleaning verification submissions from the clinical trial manufacturing area

Submission #	Standard concentration (ng/ml)[a]	%R.S.D. of the system suitability ($n = 6$)
1	6.0	2.9
2	6.0	6.3
3	6.0	4.4
4	6.0	7.1
5	5.0	1.9
6	3.0	7.9
7	3.0	6.5
8	5.0	8.0
9	3.0	4.9
10	3.0	5.5

[a] The standard concentration varies due to different APFLs used due to different sampling areas and/or different surfaces involved.

less than the lowest APFL; thus, the pre-established validation criteria were satisfied.

3.4. Long-term performance

The method for NCE 1 has been applied to clinical trial manufacturing and a total of 10 sets of swabs were submitted for cleaning verification over the course of 1 year. The precision of the standard injections utilized to demonstrate system suitability is outlined in Table 3. The R.S.D. on these injections is typically 3–6%, with an observed range of 1.9–8.0% over the course of 1 year. The data demonstrated the consistent and reliable long-term performance.

The methods developed in this paper used an ion-trap mass analyzer due to the instrument availability at the time. Although the ion-trap MS detector scan range can be minimized, it cannot collect data in selected ion monitoring (SIM) mode; therefore, it is typically not considered the first option for analyses requiring low-level detection limits or peak area precision in quantitation. However, the ion trap is capable of performing selective fragmentation (LC-MS/MS), resulting in a significant reduction of background ions. Alternatively, SIM mode from the quadrupole MS detector could be used for the signal enhancement. These approaches should significantly increase signal-to-noise ratio over the ion trap scanning (full scan) mode, thus improving upon the sensitivity and long-term performance discussed above.

Additional experiments were performed to evaluate how much improvement can be achieved in detection limits by using LC-MS/MS with an ion trap, or by using SIM on a single quadrupole. To facilitate comparison, a standard solution close to the detection limit was used. NCE 1 at 0.6 ng/ml was prepared by making a dilution with 1:1 methanol:ammonium acetate buffer, from the suitability standard solution (3 ng/ml). Six replicate injections were evaluated for each of the parent and a selected fragment ion with the ion trap, and for the selected ion with the single quadrupole, using the conditions summarized in Table 1. Table 4 summarizes the data for the signal-to-noise ratio and relative standard deviation. More than one order of magnitude

Table 4
Results summary for 0.6 ng/ml NCE 1, $n = 6$

Analyzer/ion	Average peak area	Signal-to-noise[a]	%R.S.D.
Trap/parent	37,053	3	16.5
Trap/fragment	27,749	47	8.7
Quadrupole/parent	4,386	36	1.5

[a] Peak-to-peak noise is measured.

increase in signal-to-noise was observed using the fragment ion by MS/MS, or the single quadrupole (SIM). The results provide great confidence for the further improvement of the long-term performance, or a lower APFL, when necessary.

4. Conclusion

The specific validation strategies and detection techniques pertinent to low-dose compounds were discussed. A generic LC-MS method was developed for a class of carboxylic acid compounds. The method was validated for specificity, limit of detection, recovery, precision, and stability of standard and sample solutions. In addition, the stability of the swab samples with analyte was evaluated to determine the allowable time interval between sampling CT equipment and extraction of the analyte with sample solvent. The assay-pass-fail-limit was determined to be 3 and 4 ng/ml for two new chemical entities. This value corrects for recovery of the analytes from a host of surfaces including stainless steel, anodized aluminum, Rilsan® coated aluminum, bronze, polyvinylchloride, and Oilon®. The method detection limit was calculated to be <0.5 ng/ml. The method achieved excellent precision over time on multiple injections of a standard solution at approximately 3–6 ng/ml, without the use of internal standard. The LC-MS method has proven to be rugged and reliable over the course of 1 year of clinical trials and swab submissions.

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