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Real-time endpoint monitoring and determination for a pharmaceutical salt formation process with in-line FT-IR spectroscopy

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Abstract

An application of Fourier transform infrared (FT-IR) spectroscopy equipped with an attenuated total reflectance (ATR) probe for in-line monitoring of a hydrochloride (HCl) salt formation process of 4-{1-methyl-2-piperidin-4-yl-4-[3-(trifluorometryl)phenyl]-1H-imidazol-5-yl}-*N*-[(1S)-1-phenylethyl]pyridine-2-amine (freebase), an active pharmaceutical ingredient as a P38 MAP kinase inhibitor, is described. The freebase forms both mono- and bis-HCl salts due to its structural features. The mono-HCl salt is the desired product but the bis-salt is an impurity. The key to maximizing the product yield and minimizing the impurity level is to monitor the salt-forming reaction and to terminate it at the correct HCl charge amount. The process analytical technology (PAT) provided real-time data for process control and overcame the limitations that had been previously encountered by other analytical instrumentations, such as high-performance liquid chromatography and titration. Two qualitative approaches for reaction endpoint determination were employed. In the first approach, changes in the concentration of the freebase and bis-salt were monitored via the first derivative concentration profiles. The flat point in the freebase profile and the rise in the bis-salt profile were used as a detection bracket for the endpoint of HCl charging. In the second approach, principal component analysis (PCA) was used to classify the status of the process based on a spectral library consisting of spectra collected around the endpoint. Results indicated that both methods provided adequate accuracy for endpoint control in a small window between 1.0 and 1.05 HCl to freebase mole ratio. Both methods were used to support a scaled up process. Three batches of MAP mono-HCl salt formation were successfully controlled and prepared.

Keywords: In-line FT-IR; ATR–FT-IR; Process analytics technology; Salt formation of pharmaceutical compound; Reaction endpoint determination; Principal component analysis

1. Introduction

Salt formation for an active pharmaceutical ingredient (API) is a very important step in drug development for enhancement of the API's physical properties, including water solubility, dissolution rate, chemical stability, purity, compatibility with excipients and bioavailability. An estimated half of the API molecules used in medicinal therapy are administered as salts [1]. Among all salts used in formation, HCl salts are the most commonly used for basic drug compounds. In this study, the salt-forming compound was 4-{1-methyl-2-piperidin-4-yl-4-[3-(trifluorometryl)phenyl]-1H-imidazol-5-yl}-*N*-[(1S)-1-phenylethyl]pyridine-2-amine (freebase), a P38 MAP kinase inhibitor [2]. It is a weak base with multiple basic sites and slightly different pK_a's. Two basic sites can react with HCl to form salts. The desired product is a mono-HCl salt of a secondary amine site on the piperidin ring (Fig. 1). However, the bis-salt of a secondary amine on phenylethyl is a process impurity. The reactivity of the freebase in forming the mono-salt is higher than that in forming the bis-salt, whereas the solubility of the mono-salt in the reaction medium is slightly lower than that of the bis-salt. Thus, the mono-salt product can be produced by charging HCl to the freebase batch in a controlled manner and isolating it from the reaction mixture through crystallization and filtration. A small amount of bis-salt could co-crystallize with the mono-salt if the HCl is overcharged. Therefore, it is very critical to terminate the HCl charging at the optimal point in order to get the best yield and quality for the

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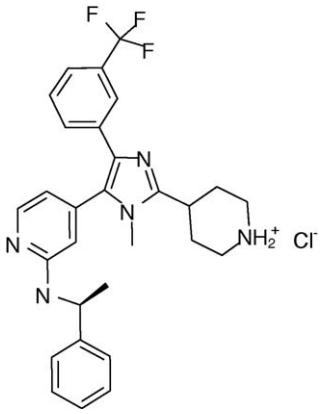

Fig. 1. Structure of the mono-HCl salt of the API.

product. The key to reach this goal is to monitor the endpoint of mono-salt formation in the reaction mixture in real-time. However, this task cannot be carried out by common analytical instrumentations such as reverse phase high-performance liquid chromatography (RP-HPLC) and titration. These instruments are able to determine the total chloride and freebase concentrations, but lack the specificity for differentiating the mono- and bis-salts. Their results can only be used in conducting a pre-estimation of the HCl charge. The accuracy of the estimation is not sufficient to ensure optimal termination of HCl charging since the yield of the mono-HCl reaction and the precipitation of mono- and bis-salts can be affected by variations in process conditions such as pH, temperature, etc. Clearly, there is a need for an on-line analytical method that is able to detect the salt formation endpoint based on direct process information, i.e. the depletion of freebase and formation of bis-salt. In addition, the on-line method is of critical importance to preventing operation errors in volume control and in concentration determination for freebase or HCl because there is no other method to detect the bis-salt once it forms.

Herein we describe a process analytical technology (PAT)-based methodology for monitoring the salt formation process. This method uses Fourier transform infrared (FT-IR) spectroscopy coupled with attenuated total reflectance (ATR) to track the depletion of freebase and the formation of bis-salt impurity. The capability of ATR–FT-IR in providing detailed, molecular level process information is the major attribute for its wide spread utilization in pharmaceutical process development which often involves complex chemistry and process procedures [3–9]. In addition, its unique feature in measuring solution concentrations of slurry samples is especially appealing to chemists and engineers working on crystallization processes. Researches in acquisition of key crystallization parameters using ATR–FT-IR and feedback control of crystallization processes have been reported [10–13]. Like these successful applications, the work reported here also takes full advantage of ATR–FT-IR. Freebase and bis-salt are distinguished by resolved peaks in the finger print region of IR spectra, and their concentrations are tracked despite the presence of mono-salt solids. One of the key aspects of this study is the development of appropriate data analysis method for endpoint detection in the absence of a suitable reference method to quantify the mono- and bis-salt concentrations. Two qualitative approaches were developed to circumvent the difficulty and used in supporting the synthesis of the preparative batches. The first is a direct concentration profiling approach. Taking advantage of high resolution of IR spectroscopy, this method uses single peaks to track the freebase and the bis-salt concentrations without the concern of spectral interference. The concentration changes are checked against a set of thresholds during the process. HCl charging is terminated once the thresholds are reached. In order to improve the robustness and sensitivity of the method, the concentration profiles are treated with first derivative to remove baselines and slops that has no process significance. The second method uses principal component analysis (PCA) to classify the process status [14–16]. In contrast to the direct concentration profiling approach, the PCA-based method uses the entire or portions of IR spectrum to track the progress of the process. Statistical algorithms are used to compress the multivariate data into a set of orthogonal vectors, i.e. principal components (PCs). Each PC represents a unique combination of the changes in the concentrations of major chemical species and other factors during a process. When a spectrum is projected into a coordinate space defined by the PCs, that spectrum will occupy a unique position according to the chemical and physical information it carries. Thus, spectra collected continuously during a process will form a trajectory in the PC space, reflecting the progress of the process. The position of the trajectory's tail (the most recent spectrum) relative to the predefined endpoint acceptance zone is constantly monitored. Once the trajectory enters the acceptance zone, the salt formation is complete and HCl charging is terminated. Results indicate that both methods provide adequate accuracy for the endpoint control in a small region between 1.0 and 1.05 equivalence of HCl in which no bis-salt should precipitate. In ideal situations, gravimetric or volumetric charge control would be appropriate for such a range. For this particular case, however, this type of control is not reliable enough since the extent of mono-salt reaction and the behavior of mono- and bis-salt precipitation are sensitive to variations in process conditions. Even the HCl charging is gravimetrically or volumetrically controlled within the range, there might still be a risk of bis-salt precipitation. This risk can be further increased if there are errors in freebase and HCl concentration determinations. On-line monitoring for terminating HCl charge based on molecular information is the best way to ensure optimal yield and quality.

2. Experiment

2.1. Reagents

The 4-{1-methyl-2-piperidin-4-yl-4-[3-(trifluorometryl) phenyl]-1H-imidazol-5-yl}-*N*-[(1S)-1-phenylethyl]pyridine hydrochloride, a P38 MAP kinase inhibitor (MAP), was synthesized by Process Research at Merck and Co. Inc. (Rahway, NJ). Hydrochloride acid (concentrated) and Isopropyl alcohol (IPA) were purchased from Fisher Scientific (Fairlawn, NJ, USA).

Deionized water was purified in house using the Millipore Milli-Q water purification system (Bedford, MA).

2.2. Instrument

The in-line FT-IR used in lab experiments was a ReactIR 1000 equipped with a Dicom^{TM} ATR probe (Mettler Toledo AutoChem, Millersville, MD). In the preparation scale reaction, the instrument was a ProcessIR made by the same company. A custom-made 36 in. long ATR probe was used to reach the reaction medium from the top of the large reactor. All spectra were recorded with a 4 cm^{-1} spectral resolution and a 2 min temporal interval. In lab experiment, 64 scans were averaged for each spectrum, whereas in the preparation scale work the number of scans were increase to 128 in order to improve the signal to noise ratio that was adversely affected by the long probe. The instrument software was interfaced with an in-house developed program written with Matlab^{TM} (The Mathworks Inc., Natic, MA). A macro in the FT-IR software saved a spectrum on the hard disk every time a new spectrum was collected. The Matlab program imported the new spectrum and performed all data analysis and display.

3. Results and discussion

3.1. Spectral peak identification

The spectra of freebase samples with different HCl addition amounts are displayed in Fig. 2. In order to increase the solubility of bis-salt for the convenience of peak identification, pure isopropyl alcohol (IPA) was used as the solvent. The peaks at 1620 and 1650 cm^{-1} correspond to mono- and bis-salts, respectively. These peaks are resulted from N–H bending vibrations. The changes of salt peaks are more dramatic when the HCl/freebase mole ratio exceeds 1.0. This may be caused by the salts' solubility increase in the presence of excessive HCl.

3.2. Endpoint determination by direct concentration profiling

In the real process, the solvent was a mixture of IPA and isopropyl acetate (IPAc). The reason to switch from pure IPA to IPA/IPAc mixture was to lower the solubility of the salts to reduce material loss to mother liquor. The bis-salt peak of 1650 cm^{-1} is at the heel of the carbonyl peak of IPAc, but is still visible when HCl is overcharged. The freebase is tracked by its peak of pyridine moiety at 1513 cm^{-1}. Because the solubility of mono-salt is low, this non-specific peak well reflects the freebase concentration. In order to improve measurement stability, the spectra were treated with second derivative, and then both freebase and bis-salt peaks were divided by the carbonyl peak of IPAc at 1735 cm^{-1}. The freebase and bis-salt profiles tracked by these peak ratios are shown in Fig. 3. Controlled HCl overcharging revealed the characteristics of the salt formation process. As HCl was charged into the batch at a constant rate, the freebase profile decreased steadily while the bis-salt profile remained low and flat, indicating precipitation of the freebase as mono-salt. When the HCl/freebase mole ratio reached 1.0, the freebase profile started to flatten since the freebase was about to be depleted. Once the mole ratio exceeded 1.0, the bis-salt profile rose quickly because a significant amount of bis-salt started to form. The sensitivity and reliability of detecting these changes can be improved by applying first derivative to the profiles. As shown by Fig. 4, the slopes in the raw profiles were removed and the changes of the profile near the end of the HCl charging became more substantial in the derivative profiles. These improvements ensured reliable detection of a 0.05 mol-equivalent of overcharged HCl, which could be translated to 5% of bis-salt as the total amount of freebase. At this level, almost all bis-salt was still in the solution and could be easily removed with the mother liquor. Therefore, the upper limit of HCl charging could be set at a 1.05 HCl/freebase mole ratio. On the other hand, the derivative profile for freebase started to move towards zero as the raw freebase profile flat-

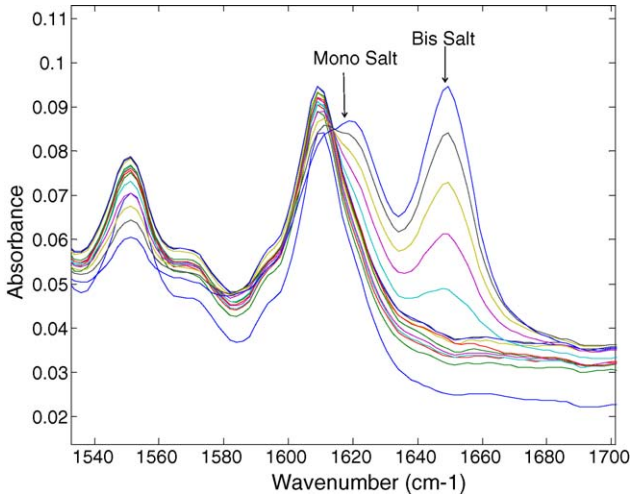

Fig. 2. FT-IR spectra of the reaction. The amine–HCl peaks for the mono- and bis-salts are identified.

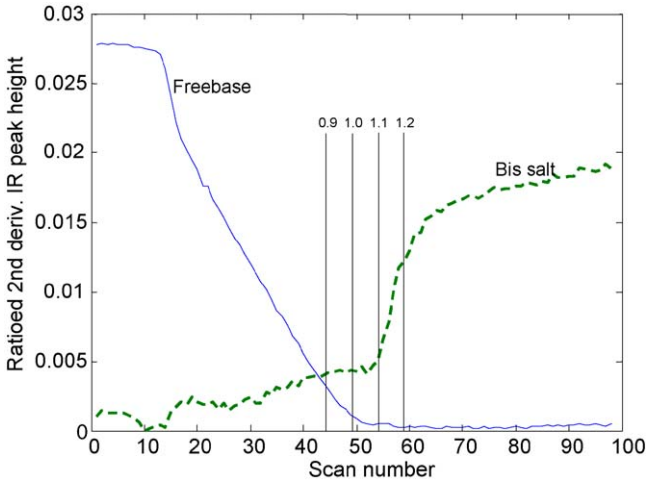

Fig. 3. The profiles of freebase (solid) and bis-salt (dash) in an experiments in which HCl was overcharged in a controlled manner. The vertical lines mark the mole ratio of HCl to total freebase.

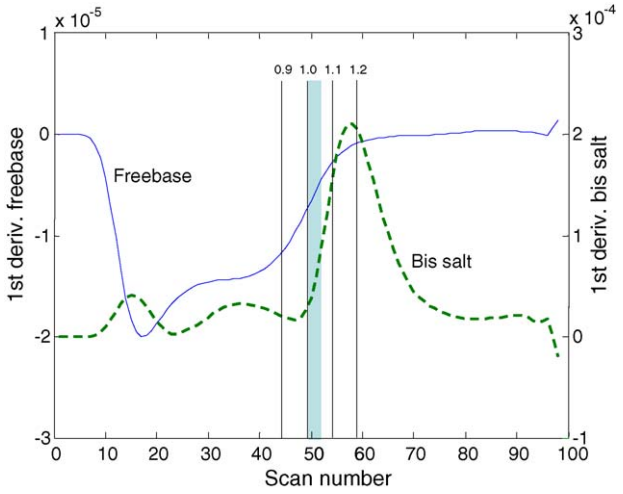

Fig. 4. The first derivative profiles of freebase (solid) and bis-salt (dash). The detection sensitivity is enhanced by using derivatives. The shaded area indicates the region of 1.0–1.05 HCl/freebase mole ratios in which the process endpoint is defined.

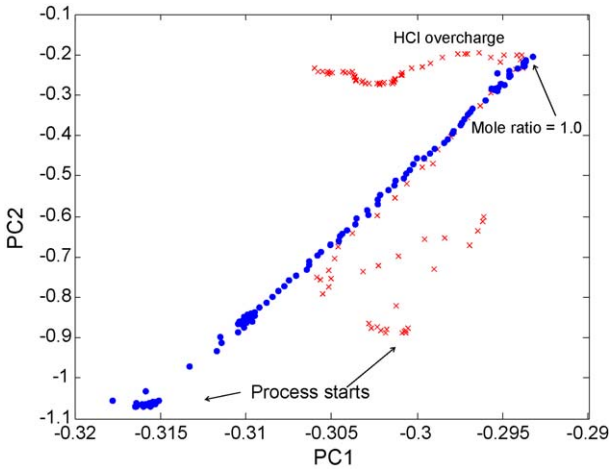

Fig. 5. The process profile tracked by PCA. The crosses represent the calibration experiment in which HCl was overcharged in a controlled manner, and the dots are for the confirmation experiment. The sharp turn of the calibration trajectory indicates HCl overcharge (>1.0 mol ratio).

tened out near the point of 1.0 HCl/freebase mole ratio. Since this occurred before any bis-salt could be detected, it was used as a lower limit for HCl charging. The upper and lower limits formed an endpoint detection bracket (see the shaded area in Fig. 4) that increased the reliability of endpoint detection. A process terminated within the endpoint bracket was ensured with the highest mono-salt yield and free of bis-salt. Two more experiments were carried out to confirm the boundaries of the bracket. The statistics and the acceptance boundaries are listed in Table 1.

Another issue need to be considered was fluctuation of the derivative profiles. As the derivative treatment removed baseline shift and other insignificant variations, it also magnified fluctuations in the profiles. A 11-point moving boxcar smoothing was applied to the profiles to reduce the fluctuations.

3.3. Endpoint determination by principal component analysis

In this work, the PC coordinates were calculated using the spectra surrounding the end of the HCl charging. Used in the calculation was the spectral region of 1488–1688 cm^{-1} which contained both freebase and bis-salt peaks. In Fig. 5, the *x*–*y* coordinates were the first PC and second PC, respectively. The crosses represented the spectra of the first experiment. The trajectory took a sharp turn when HCl overcharging started. PC1 turned from a slow increasing trend to a quick decrease from that point. The second experiment confirmed this with a very similar trajectory, represented by the dots in Fig. 5. The turning point met with the first experiment very well. It also could be seen that the PCA method gathered more information about the process than the direct concentration profiling approach did. During the first experiment, there was a temperature change. This was reflected by the zigzag of the trajectory when the temperature changed from 70 to 60 °C. The trajectory took a new course when the temperature was stabilized at 60 °C. It was overlaid by the trajectory of the second experiment which was run at a constant 60 °C. The acceptance box for the endpoint was defined by the statistics of the PC values corresponding to the region of 1.0–1.05 HCl/freebase mole ratio based on the data from three experiments. The results are listed in Table 2.

Table 1
Endpoint acceptance boundaries using freebase and bis-salt derivative profiles

Profile	HCl/freebase mole ratio	Mean (1st derivative)	S.D.	Threshold
Freebase	0.95–1.00	-6.93×10^{-6}	3.96×10^{-6}	-1.88×10^{-5}
Bis-salt	1.00–1.05	5.61×10^{-5}	5.65×10^{-6}	3.91×10^{-5}

The thresholds are the means minus three times of their respective standard deviations.

Table 2
Endpoint acceptance boundaries using principal component analysis (PCA)

Principal component	HCl/freebase mole ratio	Mean	S.D.	Low	High
PC1	1.00–1.05	−0.2945	0.0005	−0.296	−0.293
PC2	1.00–1.05	−0.2356	0.0245	−0.3091	−0.1621

The low and high boundaries are the means minus or plus their respective standard deviations.

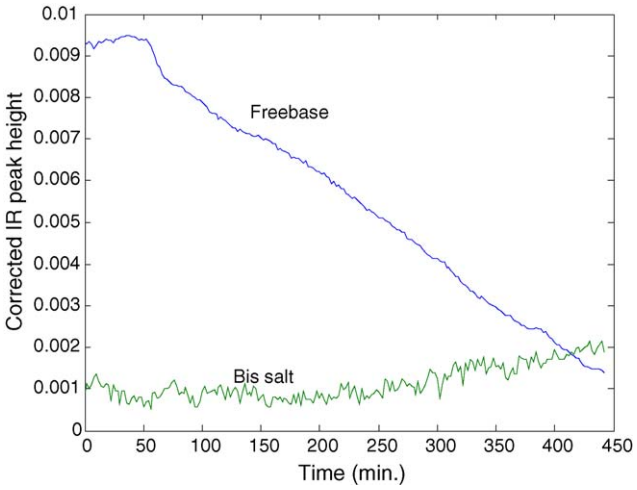

Fig. 6. Freebase and bis-salt profiles in the preparation scale process. HCl charge was at a constant rate.

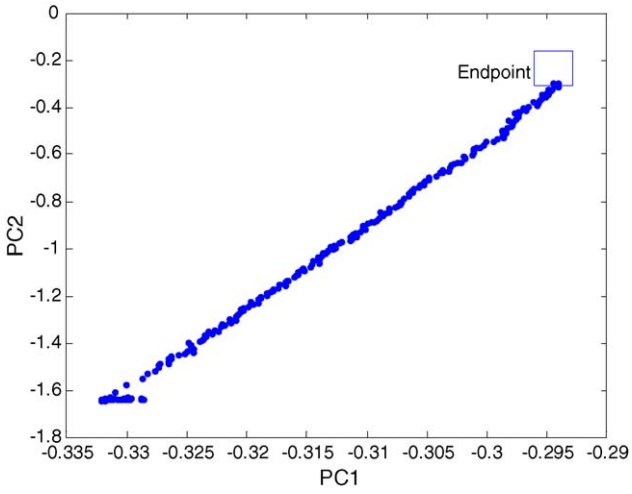

Fig. 8. PCA classification for the endpoint of the preparation scale process. The box defines the endpoint acceptance area.

3.4. Process control for preparation scale-up run

Upon the successful development and evaluation in the lab, the qualitative endpoint detection methods were used in supporting the preparation scale-up synthesis. Fig. 6 displays the concentration profiles of freebase and bis-salt for that process. During the first 60 min of HCl charging, the mono-salt built up supersaturation and started to precipitate, as indicated by a constant freebase level followed by a sudden decrease. After that, the process entered a steady state — the rate of mono-salt precipitation was equal to the rate of HCl charging, which was demonstrated by the constant decrease of freebase profile. Near the pre-estimated equimolar point, the freebase derivative profile hit the boundary (see Fig. 7). This confirmed that the endpoint had been reached and the process was consequently terminated. The endpoint confirmation requires three consecutive hits to the boundary, a measure to prevent occasional hit to the boundary and premature termination of the process. This is necessary when data are noisy. Another measure to exclude premature boundary hitting is to check the elapsed time. At early stage of a process this is easy to do. When the process is close to the end, statistical means such as the consecutive hitting requirement are necessary. The bis-salt profile, on the other hand, was flat throughout the entire process. Fig. 7 shows that its derivative profile never exceeded the limit set up by the lab experiments. The PAC endpoint detection method also confirmed that the process was terminated correctly. In Fig. 8, the reaction trajectory falls into the acceptance box and did not show the characteristic sharp turn due to HCl overcharge. All of the evidences indicate that the process has been completed successfully with the highest mono-salt yield and minimal bis-salt.

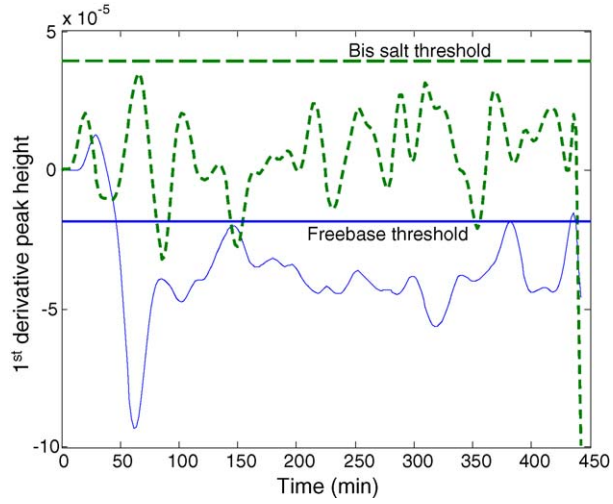

Fig. 7. The first derivative freebase (solid) and bis-salt (dash) profiles in the preparation scale process. The end of process was flagged by the freebase profile exceeding the boundary. No bis-salt was detected as its profile stayed under the boundary.

4. Conclusion

The On-line FT-IR method has demonstrated its capability to successfully monitor the endpoint of the freebase–HCl reaction for the P38 MAP kinase inhibitor. The qualitative approaches in detecting the process endpoint can be effective and accurate when the process is stable and factors that influence the measurement are minimized. Multiple experiments covering process variations are necessary to establish reliable detection thresholds or acceptance boundaries for the endpoint. In this particular case where no reference method was available to support qualitative calibration, the advantage of qualitative approaches was clearly demonstrated. In addition, the qualitative methods are more easily transferred to other instruments, as demonstrated by the use of two different FT-IR spectrometers in the lab experiments and the preparation of the scale-up reaction. This is a great advantage over quantitative calibrations in terms of saving time and resources in developing, implementing, and maintaining in-line analysis methods.

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