# Determination of Assay and Related Substances from Anhydrous Citric Acid and Citric Acid Monohydrate by Ion Chromatography

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**Abstract:** ***An accurate, simple, reproducible, and sensitive method for the estimation of citrate assay and its related substances in anhydrous citric acid and citric acid monohydrate was validated. Citrate, sulfate and oxalate ions were separated using ion chromatography technique using gradient elution with a flow rate of 1 mL/min. The linearlity of method has been tested in the range of 5.0mg/L to 50mg/L of citrate, 1.0mg/L to 20.0mg/L of sulfate and 20.0mg/L to 20.0mg/L of oxalate and correlation cofficient ($R^2$) was >0.999 for citrate, sulfate and oxalate. The method was shown excellent reproducible, linear, specific, sensitivity, rugged. The Limits of Detection and Quantification have been also established for citrate as 20µg/L & 71µg/L, for oxalate as 10µg/L & 23µmg/L and for sulfate as 8µg/L & 20µg/L respectively. Hence, the validated method is easy to adapt for regular analysis.***

**Keywords:** Citric acid, citric acid monohydrate, citrate, sulfate, oxalate, suppressed conductivity, Ion Chromatography.

## 1. Introduction

Citric acid is widely used as an excipient in the pharmaceutical industry. It is used as an antioxidant in formulations like parenteral and sublingual films.[1] It has also demonstrated to delay release of drug from enteric coated tablets or enteric coated granules.[2] Citrate buffer has provided a stabilizing effect on the photolysis of riboflavin in aqueous solution.[3] It is commonly used as a buffering agent in various analytical methods to maintain the pH of the mobile phase in the range of pH 3.0-6.2, which the range of its buffering capacity. It is also used in multiple-unit enteric-coated tablets for targeting drugs on the colon.[4]

The USP-NF monograph of anhydrous citric acid and citric acid monohydrate mentions a titrimetric method for assay with acceptance criteria of 99.5%-100.5%. Ion chromatography is more sensitive compared to titrimetry and provides better estimation of citric acid content.

The monograph also includes a test for heavy metals with a limit of 0.015% of sulfate and 0.036% of oxalic acid in the anhydrous citric acid and Citric acid monohydrate. Their provided method of qualitative analysis is based on visual inspection, which is cumbersome and less sensitive. It also involves use of hazardous and toxic chemicals like phenylhydrazine hydrochloride and hydrochloric acid. [5][6]

Thus, in the present study, a method was developed which is convenient, rapid, accurate and precise for determining the assay of citric acid along with estimation of its related substances, sulfate and oxalate from anhydrous citric acid and Citric acid monohydrate samples using Ion Chromatography with suppressed conductivity detection. Column utilized is IonPac AS19 which is an anion exchange column that allows determination of inorganic anions and oxyhalides.[7][8]

## 2. Experimental

### 2.1 Reagents and Chemicals

All chemicals used for preparation of reagents, standards and mobile phase were of analytical grade. Ultrapure deionized water (18.2 MΩ cm, Milli-Q system) was used for the preparation of mobile phase, standards and samples. Citric acid anhydrous (Qualigens Fine Chemicals) and citric acid monohydrate (Merck) was used for sample preparation; Potassium Sulfate (Merck) and Oxalic acid (Sigma Aldrich) was used for standard preparation of related substances. Potassium hydroxide (KOH) eluent was generated using Dionex EGC III KOH Potassium Hydroxide Eluent Generator Cartridge and gradient analysis was done to separate sulfate, oxalate and citric acid.

### 2.2 Apparatus

The equipment used was Thermo Scientific Dionex Integrion HPIC system having AS-AP Autosampler with a 25µL loop, IonPac AS19 column (4 x 250mm) and its guard (4 x 50mm) was used. ADRS600, 4mm suppressor at constant voltage was utilized throughout the sequence. Software used for data acquisition was Thermo Fisher Scientific Dionex Chromeleon (version: 7.20). Chromatograms were monitored simultaneously during analysis.

### 2.3 Procedure

**Preparation of Eluent:** - Dionex EGC III KOH Potassium Hydroxide Eluent Generator Cartridge was used for gradient eluent generation of 20 mM to 60 mM KOH as shown in below table (a).

**Table 1:** KOH gradient profile

| *Time, minutes* | *KOH, mM* |
|---|---|
| 0.0 | 20.0 |
| 25.0 | 60.0 |
| 25.1 | 20.0 |
| 30.0 | 20.0 |

**Preparation of standard solutions**

Citric acid standard of 1000 mg/L was prepared in ultrapure deionized water. From this 1000 mg/L standard citrate solution, 5.0, 10.0, 20.0, 30.0, 40.0, and 50.0mg/L of citrate was prepared for the Linearity study, and 6 standards of 20mg/L were prepared for the precision study. Similarly, Potassium sulfate was used for preparation of 1000 mg/L of standard sulfate solution and oxalic acid was used for the preparation of 1000 mg/L of standard oxalate solution. For the linearity study of related substances in citric acid, 1.0, 2.0, 4.0, 8.0, 16.0 and 20.0 mg/L of sulfate from the above standard sulfate solution and 2.0, 4.0, 6.0, 10.0, 15.0, and 20.0mg/L of oxalate from above standard oxalate solution was added to 5.0, 10.0, 20.0, 30.0, 40.0, and 50.0mg/L of citrate prepared from standard citrate solution.

**Sample preparation**

For assay of Anhydrous Citric acid and citric acid monohydrate sample: weight of sample equivalent to 100 mg of citrate was dissolved in 100 ml of ultrapure deionized water to provide a solution containing 1000 mg/L of citrate. From this solution, 0.2 ml was taken and further diluted to 10 ml providing a 20mg/L solution.

For related substances in Anhydrous Citric acid and Citric acid Monohydrate sample: Around 200mg of sample was mixed with 10 ml of ultrapure deionized water.

An Autosampler (Dionex) was used to inject standard solution containing citrate, sulfate and oxalate ions into the ion chromatography system. Subsequently, the standard solution in the sample loop was transferred onto the separator column, on which citrate, and its related substances, sulfate and oxalate were separated. A sequence containing the blank, standards, samples and recovery samples were run and results were then interpreted.

## 3. Results and Discussions

**Limit of Detection and Limit of Quantification:**

Limit of Detection (LOD) for Citrate was 20 µg/L and observed signal to noise ratio (S/N) was 3. LOD for sulfate was 8 µg/L and observed signal to noise ratio (S/N) was 3. LOD for oxalate was 10 µg/L and observed signal to noise ratio (S/N) was 4. Limit of Quantification (LOQ) for citrate was 71 µg/L and observed signal to noise ratio (S/N) was 10. Similarly, LOQ for sulfate was 20 µg/L, and observed signal to noise ratio (S/N) was 12 and LOQ for oxalate was 23 µg/L, and observed signal to noise ratio (S/N) was 11. Table 2 shows results for LOD and LOQ of citrate, sulfate and oxalate

**Table 2:** LOD and LOQ data for citrate, sulfate and oxalate

| *Citrate* | *Amount, µg/L* | *S/N* |
|---|---|---|
| LOD | 20 | 3 |
| LOQ | 71 | 10 |

| *Sulfate* | *Amount, µg/L* | *S/N* |
|---|---|---|
| LOD | 8 | 3 |
| LOQ | 20 | 12 |

| *Oxalate* | *Amount, µg/L* | *S/N* |
|---|---|---|
| LOD | 10 | 4 |
| LOQ | 23 | 11 |

Linearity and Range:

The response of citrate was linear over the range of 5.0 to 50.0mg/L, sulfate was linear over the range of 1 to 20 mg/L, and oxalate was linear over the range of 2 to 20 mg/L. Calibration curve fits well and that is significantly linear having correlation coefficient of 0.999 for citrate, 0.999 for oxalate and 0.9971 for sulfate. Each standard injection was repeated thrice. Therefore, number of calibration points (n) for linearity study was 18. Its data had been shown in table 3.

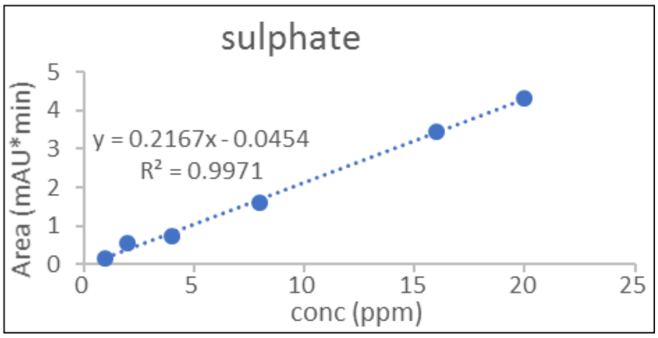

**Figure 1:** Linearity plot for Sulfate

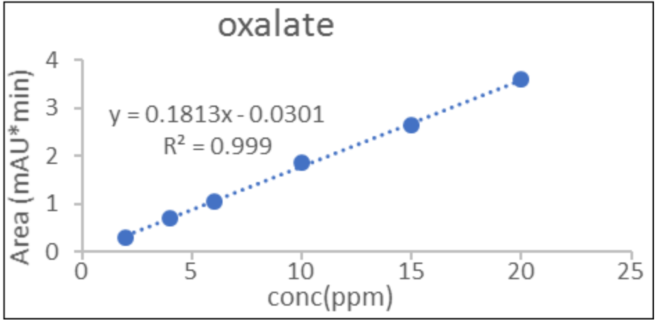

**Figure 2:** Linearity plot for Oxalate

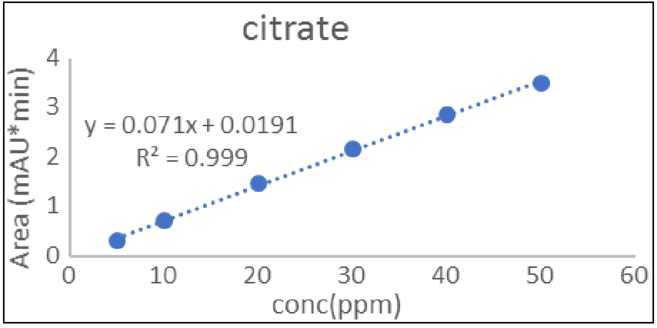

**Figure 3:** Linearity plot for Citrate

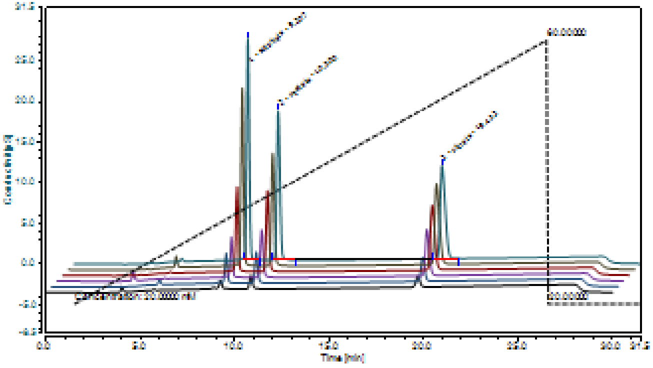

**Figure 4:** Overlaid Linearity plot for Citrate, sulfate and oxalate

**Table 3:** Linearity data for citrate sulfate and oxalate

| *Analyte* | *Points* | *Corr. Coeff.* | *Offset* | *Slope* |
|---|---|---|---|---|
| Citrate | 6 | 0.9990 | 0.0145 | 0.0711 |
| Sulfate | 6 | 0.9971 | -0.0454 | 0.2167 |
| Oxalate | 6 | 0.9991 | -0.0284 | 0.1807 |

Specificity:
Method specificity was also done with separate injection of citrate (10mg/L), sulfate (10mg/L) and oxalate (10mg/L). Its chromatogram was shown in figure 5.

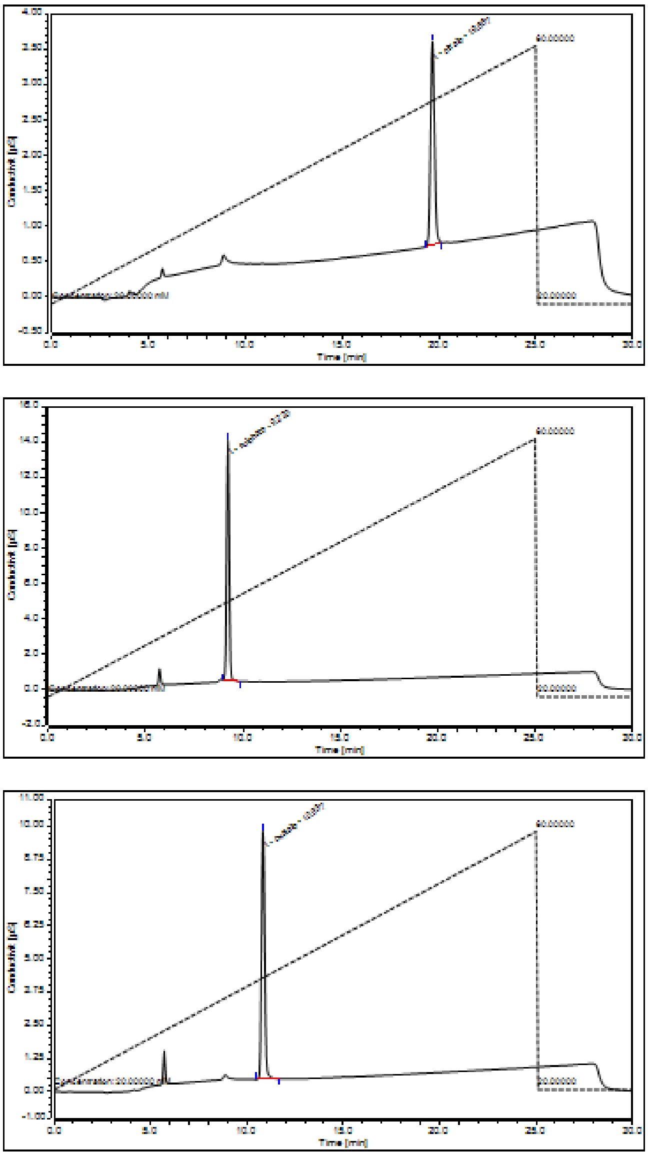

**Figure 5:** Specificity chromatograms for citrate, sulfate and oxalate (10.0mg/L).

Replicate injections of mixture of citrate, sulfate and oxalate were done and their percent relative standard deviation for peak area was 0.72, 0.93, and 0.58% respectively. Table 4 shows results for its precision study.

**Table 4:** Precision data for citrate, sulfate and oxalate

| *Analyte* | *Amount, mg/L* | *% RSD (n=6)* |
|---|---|---|
| Citrate | 20.0 | 0.72 |
| Sulfate | 4.0 | 0.93 |
| Oxalate | 6.0 | 0.58 |

Chromatogram for citrate, sulfate and oxalate standard mixture for six consecutive injections is shown in figure 6.

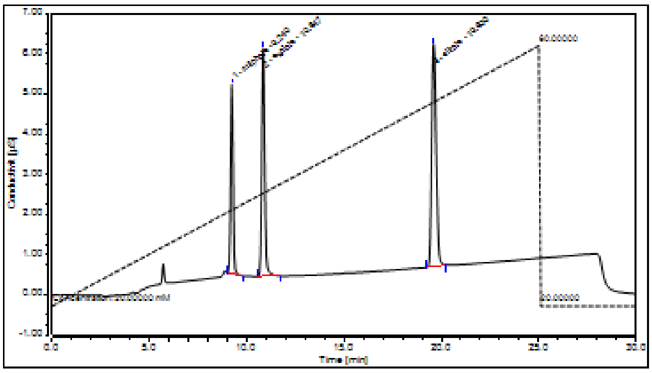

**Figure 6:** Standard chromatograms Citrate (20.0 mg/L), sulfate (4.0 mg/L) and oxalate (6.0 mg/L) for precision (n=6)

## 4. Sample Results

For assay of citric acid in samples, Six replicates injections of both samples were injected and their %RSD was calculated. Table 5 shows results of precision in sample and Table 6 shows percentage assay of citric acid and citric acid monohydrate

**Table 5:** Results of precision of citric acid samples

| Sample | B.No. | %RSD |
|---|---|---|
| Anhydrous citric acid | NL35726110S | 0.09 |
| Citric acid monohydrate | DH7D671741 | 0.12 |

**Table 6:** Assay results of citric acid content in samples

| *Sample* | *B.No.* | *Assay (% content)* |
|---|---|---|
| Anhydrous citric acid | NL35726110S | 100.37 |
| Citric acid monohydrate | DH7D671741 | 100.43 |

Citric acid and citric acid monohydrate percentage assay was passing and it is within USP monograph limit. Accuracy: Percentage recovery was determined by spiking standard citrate at three levels 50,100 and 150%) in citric acid sample containing known concentration (20 mg/L) of citrate.

**Table 7:** Results of Recovery of citrate in samples

| For Anhydrous citric acid (B.No. NL35726110S) | | | | |
|---|---|---|---|---|
| *Recovery Level* | *Target Concentration* | *Amount Added mg/L* | *Amount Recovered mg/L* | *% Recovery ± Std.Dev.* |
| 1 | 50% | 10 | 10.60 | 102.02±0.30 |
| 2 | 100% | 20 | 20.20 | 100.51± 0.04 |
| 3 | 150% | 30 | 28.72 | 97.45± 2.68 |
| For Citric acid monohydrate (B.No. NL35726110S) | | | | |
| *Recovery Level* | *Target Concentration* | *Amount Added mg/L* | *Amount Recovered mg/L* | *% Recovery ± Std.Dev.* |
| 1 | 50% | 10 | 10.45 | 101.50± 0.64 |
| 2 | 100% | 20 | 20.30 | 100.76± 0.60 |
| 3 | 150% | 30 | 30.27 | 100.54± 1.25 |

For related substances in citric acid and citric acid monohydrate:

Six replicates of both samples were prepared by dissolving 0.2 g in 10 mL of citrate and their percent relative standard deviation (%RSD) was calculated. Table 8 and 9 show results of precision results of sulfate and oxalate in samples

**Table 8:** Precision data for anhydrous citric acid sample (B.No. NL35726110S)

| *Analyte* | *Number of injections* | *% RSD* |
|---|---|---|
| Sulfate | 5 | N.D. |
| Oxalate | 5 | 1.66% |

**Table 9:** Precision data for Citric acid monohydrate sample (B.No. DH7D671741)

| *Analyte* | *Number of injections* | *% RSD* |
|---|---|---|
| Sulfate | 4 | N.D. |
| Oxalate | 4 | 0.91% |

Recovery:
For recovery study, sample was spiked with standard sulfate and oxalate at three different levels as shown in below table. Recovery test solutions were injected in triplicate.

**Table 10:** Recovery study (sulfate and oxalate) for Anhydrous citric acid sample (n = 3) (B.No. NL35726110S)

| For Sulfate | | | | |
|---|---|---|---|---|
| Recovery Level | Target Concentration | Amount Added mg/L | Amount Recovered mg/L | % Recovery ± Std.Dev. |
| 1 | 50% | 2 | 2.0197 | 100.98±1.48 |
| 2 | 100% | 4 | 3.8989 | 97.47± 0.62 |
| 3 | 150% | 8 | 7.8565 | 98.20± 0.71 |
| For Oxalate | | | | |
| Recovery Level | Target Concentration | Amount Added mg/L | Amount Recovered mg/L | % Recovery ± Std.Dev. |
| 1 | 50% | 4 | 4.11 | 102.97± 1.27 |
| 2 | 100% | 6 | 6.25 | 104.29± 0.68 |
| 3 | 150% | 10 | 10.39 | 103.98± 0.75 |

**Table 11:** Recovery study (sulfate and oxalate) for Citric acid monohydrate sample (n = 3) (B.No. DH7D671741)

| *For Sulfate* | | | | |
|---|---|---|---|---|
| *Recovery Level* | *Target Concentration* | *Amount Added* mg/L | *Amount Recovered mg/L* | *% Recovery* ± *Std.Dev.* |
| 1 | 50% | 2 | 2.0167 | 100.83± *4.09* |
| 2 | 100% | 4 | 4.0200 | 100.50± *0.30* |
| 3 | 150% | 8 | 8.0528 | 100.66± *0.43* |
| *For Oxalate* | | | | |
| *Recovery Level* | *Target Concentration* | *Amount Added mg/L* | *Amount Recovered mg/L* | *% Recovery* ± *Std.Dev.* |
| 1 | 50% | 4 | 4.1196 | 102.99± *1.08* |
| 2 | 100% | 6 | 6.1085 | 101.80± *0.51* |
| 3 | 150% | 10 | 10.4533 | 104.53± *0.45* |

Since the method was proved to be precise and accurate for determination of sulfate and oxalate, it was used to determine their content in samples. As per USP monograph of citric acid and citric acid monohydrate, there is limit test for sulfate as not more than (NMT) 0.015% (150 mg/Kg) and Oxalate not more than (NMT) 0.036% (360 mg/Kg).

**Table 12:** Routine sample analysis results for sulfate and oxalate content in samples

| *Sample* | *B.No.* | *Sulfate mg/Kg* | *Oxalate mg/Kg* |
|---|---|---|---|
| Anhydrous Citric acid | NL35726110S | Not Detected | 17.75 |
| Citric acid Monohydrate | DH7D671741 | Not Detected | 18.34 |

All samples were passing for its sulfate and oxalate limit. Sample Chromatogram was shown is figure 7 and figure 8.

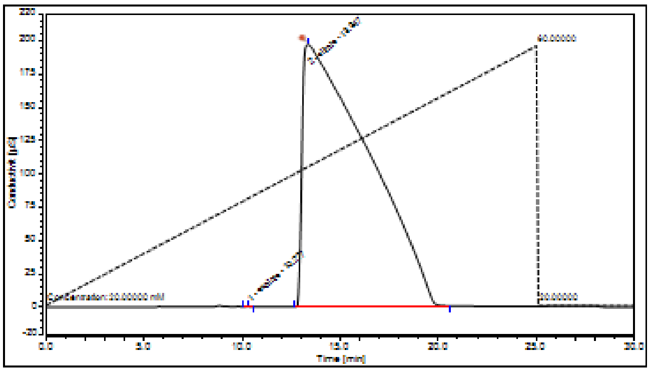

**Figure 7:** Anhydrous citric acid sample chromatogram (B. No. NL35726110S) for sulfate and oxalate estimation.

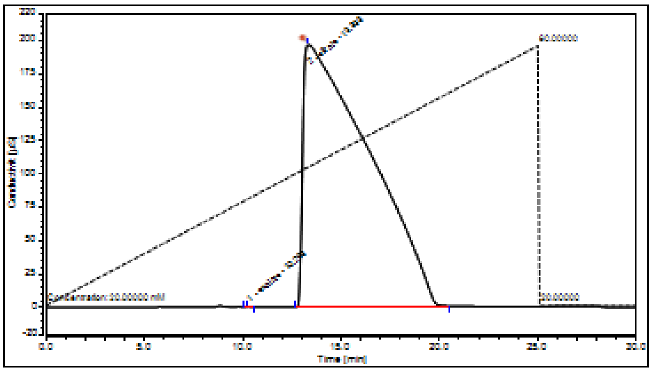

**Figure 8:** Citric acid monohydrate sample chromatogram (B. No. DH7D671741) for sulfate and oxalate estimation.

Same method was used on another Ion Chromatography instrument (ICS 5000+) with another IonPac AS19 column, for which there is no significant variation of sample results were observed.

## 5. Conclusions

Ion Chromarography with suppressed conductivity detection gives specific, sensitive and precise method for estimation of sulfate and oxalate in citric acid samples. This present method was used for analysis of anhydrous citric acid and Citric acid monohydrate for their citrate, sulfate and oxalate content without any pretreatment. The detection limits for citrate was 20.0µg/L, for oxalate was 10.0µg/L and for sulfate was 8µg/L. This technique is cost-effective with respect to analysis required for keeping a check on the limits of sulfate and oxalate as provided by USP monograph and other regulatory bodies. This method can also be useful for checking assay of citrate and its impurities/related substances from these samples.

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## Author Profile

**Dr. Chetan Chavan** had completed the B.Sc (Chemistry). and M.Sc (Chemistry) degrees from Mumbai University and completed his ph.D in year, 2018., He had joined Dionex India Pvt. Limited, which is now Thermo Fisher Scientific Pvt. Ltd., as Applications Manger for Ion Chromatography (IC) and HPLC and currently working as Product Manager – IC/SP. His role is to provide technical support coordination with factory for India team requirements, provide guidance to sales, application and service team for IC and HPLC.

**Mr. Chanakya Thaker** received the B. Sc (Chemistry) and M.Sc (Analytical Chemistry) degrees from Mumbai University in 2006 and 2008, respectively. From 2009, he had joined Dionex India Pvt. Limited, which is now Thermo Fisher Scientific Pvt. Ltd., as Applications Specialist for Ion Chromatography (IC) and HPLC and currently working as Applications Manager – IC/SP. His role is to manage application team, provide customer training, and sample analysis, technical presentations, method development and troubleshooting on IC and HPLC systems.

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**Ms. Jyotsna Soneji** has completed M. Pharm degree from Mumbai University in 2018 and received B. Pharm degree from Mumbai University in 2016. She has joined Thermo Fisher Scientific in 2018 as a Management trainee in Chromatography and Mass Spectrometry division. Her role is to provide sample analysis, method development and troubleshooting on IC systems.