

Automated Sample preparation for Toothpaste/Gel includes the determination of Fluoride ions in 1:10 and 1:100 Dilutions, the determination of Total and Free Fluoride ions in MFP containing toothpaste, determination of potassium, and determination of Triclosan.

**Toothpaste –Manual vs. Robotic**

**FLUORIDE (at 1100ppm)**

| Sample# | Manual | Robotic      |
|---------|--------|--------------|
| 1       | 1118   | 1122         |
| 2       | 1104   | 1112         |
| 3       | 1118   | 1100         |
| 4       | 1120   | 1100         |
| 5       | 1105   | 1116         |
| Mean    | 1113   | 1110(99.7%)* |
| RSD %   | 0.70 % | 0.88 %       |

\*Expressed as a percentage of the manual results

**Total Soluble F in MFP Containing TP**

| Extra Whitening Toothpaste<br>Total Soluble Fluoride (in ppm's), Theo.=1056ppm |                                                                       |                                                                          |                                                                    |                                                                  |                                                                    |
|--------------------------------------------------------------------------------|-----------------------------------------------------------------------|--------------------------------------------------------------------------|--------------------------------------------------------------------|------------------------------------------------------------------|--------------------------------------------------------------------|
| Sample Prep                                                                    | Ion Selective Electrode Method                                        | Ion Chromatography Method<br>By Dionex DX500 with Eluent Generator EG-40 |                                                                    |                                                                  |                                                                    |
|                                                                                | Manual                                                                | Manual                                                                   |                                                                    | Robotic                                                          |                                                                    |
|                                                                                | By Acid Hydrolysis<br>Then determined by F<br>Ion Selective Electrode | Peak Area<br>By Dionex Ion<br>Chromatography with AS<br>15 Column        | Peak Height<br>By Dionex Ion<br>Chromatography with<br>AS15 Column | Peak Area<br>By Dionex Ion<br>Chromatography with<br>AS15 Column | Peak Height<br>By Dionex Ion<br>Chromatography with<br>AS15 Column |
| 1                                                                              | 1019                                                                  | 1346                                                                     | 1100                                                               | 1077                                                             | 1072                                                               |
| 2                                                                              | 1022                                                                  | 1344                                                                     | 1113                                                               | 1045                                                             | 1037                                                               |
| 3                                                                              | 1026                                                                  | 1339                                                                     | 1074                                                               | 1019                                                             | 1045                                                               |
| 4                                                                              | 1034                                                                  | 1340                                                                     | 1058                                                               | 1051                                                             | 1055                                                               |
| 5                                                                              | 1034                                                                  | 1344                                                                     | 1062                                                               | 1044                                                             | 1045                                                               |
| Mean                                                                           | 1026                                                                  | 1047(102.7%)                                                             | 1081(106.0%)                                                       | 1051(100.4%)                                                     | 1050(97.3%)                                                        |
| %RSD                                                                           | 0.42%                                                                 | 0.09%                                                                    | 2.2%                                                               | 1.4%                                                             | 1.3%                                                               |

**Triclosan in Toothpastes**

| Formula<br>A | Triclosan (%w/w)<br>Theo.(0.300%) |          | Formula<br>B | Triclosan (%w/w)<br>Theo.(0.300%) |         |
|--------------|-----------------------------------|----------|--------------|-----------------------------------|---------|
|              | Manual                            | Robotic  |              | Manual                            | Robotic |
| Sample#      |                                   |          | Sample#      |                                   |         |
| 1            | 0.286                             | 0.289    | 1            | 0.303                             | 0.303   |
| 2            | 0.290                             | 0.287    | 2            | 0.296                             | 0.305   |
| 3            | 0.289                             | 0.292    | 3            | 0.302                             | 0.292   |
| 4            | 0.283                             | 0.290    | 4            | 0.305                             | 0.297   |
| 5            | 0.285                             | 0.293    | 5            | 0.292                             | 0.300   |
| Mean=        | 0.287                             | 0.290    | Mean=        | 0.300                             | 0.299   |
| %RSD=        | 1.0%                              | * 101.0% | %RSD=        | 1.8%                              | *99.7%  |

\*Expressed as a percentage of the manual results

**Conclusion**

The answer for us was automation using the Prelude Workstation. Workload is constantly increasing and products are becoming more complex with new compounds. There is continuous pressure to reduce cycle time, cut costs and improve productivity. The use of robotic automation has increased productivity; improved accuracy and precision of analytical data all with a system meeting 21 CFR Part 11 compliance.



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**The Use of Automation in Product Analysis**

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**Introduction**

There are various reasons to consider automation in Product Analysis. Scientific reasons include increasing assay precision, and accuracy, ability to walk-a-way from the system performing the assay to work on other tasks, and finally to determine analytes with state of the art equipment. Economic reasons would be to realize labor savings, while proving faster turn around in cycle time; all while performing the analysis on systems that are 21 CFR Part 11 Compliant -ready.

The Benefits are numerous; reduction of human errors; minimization of out of specification determinations, increase in productivity with analyst performing other critical tasks, cycle time reduction, and cost savings from time on task, reagents and disposables.

We will discuss the Method development, validation and summary of difficult samples for analysis.



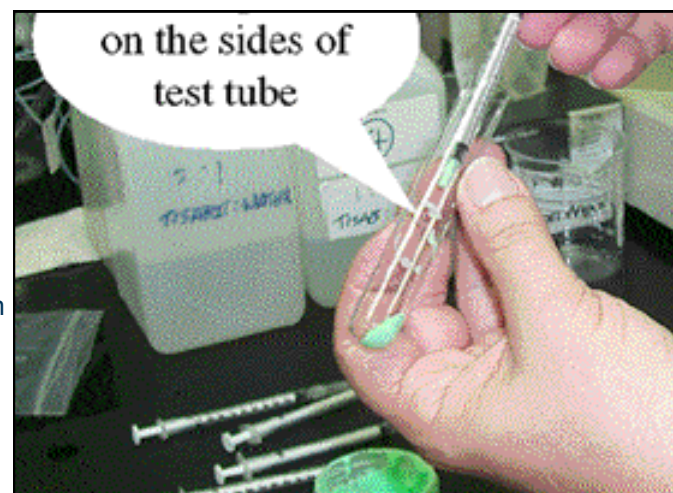
## Method Development

### Sample Delivery

Our samples consist primarily of toothpaste and gel products. It is important to introduce the sample to the bottom of the test tube for better dispersion. Due to the high viscosity; sample introduction of gels and toothpaste can be difficult leaving sample adhering to the wall of the test tube. The amount of sample in our case is 0.10-0.60mL. We tried several techniques using spatulas, Pasteur pipettes and syringes.

To improve this, we chose Supelco™ Disposable flow Control Valve Lines(57059) and connected this to a BD 1cc Slip tip plastic syringe.

To delivery the sample, we filled the 1cc syringe with toothpaste; then delivered exactly 0.55mL into the bottom of the test tube without touching the side of the test tube



### Sample Dispersion Discussion

Toothpaste comes in different formulations with varying densities; the amount and type of solid abrasive can vary by product. To disperse our sample; we added 20 - 5mm glass beads to the bottom of the tube; then delivering our sample to the top of the beads.



The Prelude® Workstation offers vortex mixing at 4 variable speeds with a settable timer, selectable volume, with the number of times chosen by the analyst based on the method. We found that we achieved adequate dispersion based on visible inspection at speed 2 for 15 minutes.

### Dilution-Ratiometric Additions

Previously we relied on volumetric dilutions. Gravimetric dilution determinations are performed at every step during the sample preparation and documented in a Microsoft Excel Spreadsheet.

### Gravimetric Confirmations of every step of the Spl. Preparation

| Tube No. | Lot #   | Sample ID      | Wt (g) | Car Disp (g) | First Dilution Ratio (g:50) | Car Asp (g) | Car Disp (g) | Second Dilution Ratio (19.99:20.00) | Car Asp (g) | Car Disp (g) | Third Dilution Ratio (19.99:20.00) | Total Dilution Ratio (1999:2000) |
|----------|---------|----------------|--------|--------------|-----------------------------|-------------|--------------|-------------------------------------|-------------|--------------|------------------------------------|----------------------------------|
| 1        | Control |                | 0.1096 | 5.4814       | 50.0                        | 0.4209      | 8.6269       | 19.99                               | 0.424       | 8.633        | 20.00                              | 200.00                           |
| 2        | 244     | P1512.20/0M    | 0.1205 | 5.0363       | 50.0                        | 0.4223      | 8.6561       | 19.99                               | 0.423       | 8.663        | 20.00                              | 199.92                           |
| 3        | 246     | P1544.20/12M   | 0.1229 | 5.1371       | 50.0                        | 0.4248      | 8.8087       | 20.00                               | 0.4221      | 8.6499       | 20.02                              | 199.99                           |
| 4        | 261     | P1544.20/12M   | 0.1027 | 5.1379       | 50.0                        | 0.4287      | 8.8216       | 20.00                               | 0.4235      | 8.6823       | 20.02                              | 200.44                           |
| 5        | 269     | P1594.20/0M    | 0.1243 | 5.2009       | 49.9                        | 0.4287      | 8.8248       | 19.99                               | 0.4243      | 8.6929       | 20.02                              | 199.92                           |
| 6        | 270     | C11330.20/12M  | 0.1081 | 5.3984       | 50.0                        | 0.4287      | 8.8142       | 20.00                               | 0.4225      | 8.6653       | 20.02                              | 200.62                           |
| 7        | 272     | P1199.20/24M   | 0.1117 | 5.8851       | 50.0                        | 0.4281      | 8.8231       | 19.99                               | 0.4235      | 8.681        | 20.03                              | 200.24                           |
| 8        | 273     | P1199.20/24M   | 0.1133 | 5.658        | 50.0                        | 0.4265      | 8.8406       | 19.99                               | 0.4232      | 8.6716       | 20.02                              | 199.94                           |
| 9        | 274     | C1199.40/1.5M  | 0.1045 | 5.235        | 50.0                        | 0.4241      | 8.4952       | 19.99                               | 0.424       | 8.6922       | 20.02                              | 199.99                           |
| 10       | Control |                | 0.1159 | 5.735        | 50.0                        | 0.4243      | 8.4995       | 19.99                               | 0.4245      | 8.697        | 20.02                              | 200.01                           |
| 11       | 275     | P1541.20/12M   | 0.1089 | 5.4376       | 50.0                        | 0.4235      | 8.4873       | 19.99                               | 0.4247      | 8.6961       | 20.02                              | 199.91                           |
| 12       | 276     | P1593.20/0M    | 0.1129 | 5.6451       | 50.0                        | 0.425       | 8.5094       | 19.99                               | 0.4252      | 8.5082       | 20.01                              | 200.19                           |
| 13       | 277     | P1592.20/0M    | 0.1102 | 5.6001       | 49.9                        | 0.4242      | 8.4838       | 19.97                               | 0.424       | 8.6816       | 20.02                              | 199.59                           |
| 14       | 278     | P1547.20/12M   | 0.119  | 5.8419       | 50.0                        | 0.4261      | 8.6366       | 19.99                               | 0.424       | 8.6894       | 20.02                              | 199.91                           |
| 15       | 278     | P1543.20/12M   | 0.1191 | 5.8522       | 50.0                        | 0.4268      | 8.6449       | 19.99                               | 0.4242      | 8.6926       | 20.02                              | 200.09                           |
| 16       | 280     | P1529.20/12M   | 0.1037 | 5.1030       | 50.0                        | 0.4248      | 8.4931       | 19.99                               | 0.4240      | 8.5103       | 20.04                              | 200.34                           |
| 17       | 281     | C11244.40/1.2M | 0.1095 | 5.4715       | 50.0                        | 0.426       | 8.5258       | 19.99                               | 0.4241      | 8.6822       | 20.01                              | 199.91                           |
| 18       | 282     | P1403.20/0M    | 0.1184 | 5.9131       | 50.0                        | 0.4295      | 8.5122       | 19.99                               | 0.4245      | 8.6957       | 20.01                              | 199.83                           |
| 19       | 282     | P1403.20/0M    | 0.1222 | 5.1017       | 50.0                        | 0.4249      | 8.5096       | 20.00                               | 0.424       | 8.6878       | 20.02                              | 199.95                           |
| 20       | Control |                | 0.1159 | 5.7932       | 50.0                        | 0.4254      | 8.5097       | 19.97                               | 0.4251      | 8.5117       | 20.02                              | 199.97                           |
| 21       | 283     | P1199.20/12M   | 0.1126 | 5.6341       | 50.0                        | 0.4262      | 8.5333       | 19.99                               | 0.4243      | 8.6917       | 20.01                              | 200.24                           |
| 22       | 284     | P1594.20/0M    | 0.1205 | 5.0256       | 50.0                        | 0.4263      | 8.5331       | 19.99                               | 0.4249      | 8.5112       | 20.03                              | 200.09                           |
| 23       | 284     | P1594.20/0M    | 0.1101 | 5.5222       | 50.0                        | 0.4259      | 8.5284       | 19.99                               | 0.4246      | 8.5814       | 20.03                              | 200.15                           |
| 24       | 285     | P1404.20/0M    | 0.1105 | 5.5223       | 50.0                        | 0.4287      | 8.5254       | 19.99                               | 0.4241      | 8.6861       | 20.01                              | 199.95                           |
| 25       | 285     | P1404.20/0M    | 0.0999 | 4.9826       | 50.0                        | 0.4241      | 8.4959       | 19.99                               | 0.4236      | 8.6826       | 20.03                              | 200.11                           |
| 26       | 286     | P1199.20/24M   | 0.1035 | 5.1348       | 50.0                        | 0.4248      | 8.5013       | 19.99                               | 0.424       | 8.6964       | 20.04                              | 200.55                           |
| 27       | 286     | P1199.20/24M   | 0.1013 | 5.0619       | 50.0                        | 0.4269      | 8.5094       | 19.99                               | 0.4259      | 8.5127       | 20.02                              | 200.01                           |
| 28       | 287     | P1595.20/0M    | 0.1019 | 5.0974       | 50.0                        | 0.4241      | 8.4993       | 19.99                               | 0.4237      | 8.693        | 20.04                              | 200.37                           |
| 29       | 287     | P1595.20/0M    | 0.1021 | 5.1093       | 50.0                        | 0.4241      | 8.5099       | 19.99                               | 0.4242      | 8.6923       | 20.00                              | 199.77                           |
| 30       | Control |                | 0.115  | 5.9016       | 50.0                        | 0.425       | 8.5081       | 19.99                               | 0.424       | 8.6945       | 20.03                              | 200.38                           |
| 31       | 289     | P1191.20/24M   | 0.1151 | 5.7529       | 50.0                        | 0.4258      | 8.5203       | 19.99                               | 0.4242      | 8.6943       | 20.02                              | 200.12                           |
| 32       | 289     | P1556.20/12M   | 0.1015 | 5.0754       | 50.0                        | 0.4246      | 8.4987       | 19.99                               | 0.4236      | 8.6848       | 20.03                              | 200.28                           |
| 33       | 290     | P1593.20/0M    | 0.1235 | 5.1756       | 50.0                        | 0.4282      | 8.5192       | 20.00                               | 0.4241      | 8.6919       | 20.02                              | 200.24                           |
| 34       | 290     | P1593.20/0M    | 0.1224 | 5.1166       | 50.0                        | 0.4243      | 8.4972       | 19.99                               | 0.4274      | 8.6719       | 20.02                              | 200.15                           |
| 35       | 291     | P1556.20/12M   | 0.1113 | 5.5623       | 50.0                        | 0.4284      | 8.523        | 19.99                               | 0.4236      | 8.6849       | 20.03                              | 200.22                           |
| 36       | 292     | P1184.20/24M   | 0.1012 | 5.0811       | 50.0                        | 0.4285      | 8.5289       | 20.00                               | 0.4248      | 8.6866       | 20.02                              | 200.24                           |
| 37       | 293     | P1183.20/24M   | 0.1005 | 5.0386       | 50.0                        | 0.4262      | 8.5318       | 19.99                               | 0.4249      | 8.5137       | 20.04                              | 200.54                           |
| 38       | 294     | P1597.20/0M    | 0.1024 | 5.1193       | 50.0                        | 0.4203      | 8.5074       | 19.97                               | 0.4219      | 8.6409       | 20.01                              | 199.97                           |
| 39       | 294     | P1597.20/0M    | 0.0989 | 4.9398       | 50.0                        | 0.4256      | 8.5192       | 19.99                               | 0.4235      | 8.6898       | 20.04                              | 200.37                           |
| 40       | Control |                | 0.1197 | 5.9843       | 50.0                        | 0.426       | 8.5396       | 20.01                               | 0.4237      | 8.6954       | 20.04                              | 200.37                           |
| 41       | 298     | P1182.20/24M   | 0.1009 | 5.292        | 50.0                        | 0.4249      | 8.5169       | 20.01                               | 0.4282      | 8.5289       | 20.04                              | 200.63                           |

The next step in our method is Sample Filtration. Once the solids have settled in the bottom of the tube; the system allows a Pre-Wet volume to be selected; then the next ratiometric addition can be made after filtration. Carryover studies were performed and were less than 1%

### Analytical Test Results

|                                  | Manual  | Robotic |
|----------------------------------|---------|---------|
| • Sample Prep Time               | 120 min | 40 min  |
| • Solvent used & Waste generated | 150 mL  | 30 mL   |

### Validation

Acceptance criteria to determine equivalence of the two method was demonstrated by comparing the results from 5 replicate samples prepared manually and by the Prelude Workstation. No greater than 2.9% difference between means of the manual and the automated method is acceptable. The maximum relative Standard Deviation(%RDS) should be less than 4.7% for 5 replicates( 4.2% for 6) As per SOP (Demonstrating Lab to Lab Equivalence)