বাংলাদেশ স্ট্যান্ডার্ডস এন্ড টেস্টিং ইনস্টিটিউশন
শিল্প মন্ত্রণালয়
গণপ্রজাতন্ত্রী বাংলাদেশ সরকার
মান ভবন, ১১৬/ক, তেজগাঁও শিল্প এলাকা, ঢাকা-১২০৮।
ফোন: ৮৮৭০২৭৫, ৮৮৭০২৭৭, ৮৮৭০২৭৮, ৮৮৭০২৭৯, ৮৮৭০২৮০, ৮৮৭০২৮১, ৯১৩১৫৮২
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মুজিববর্ষের দর্শন
টেকসই শিল্পায়ন।

Research Completion Report

Research Proposal:

Development of an improved method in BSTI for analysis of Benzoic Acid and Sorbic Acid for fruits and vegetable products.

Submitted by:

Md. Shahed Reza, Assistant Director (Chemical) and
Shifat Sharmin, Senior Examiner (Chemical)

Bangladesh Standards and Testing Institution (BSTI)

Duration: 14.03.2021 to 14.12.2021

Table of Content

Research Proposal

Sl. No. & Title	Description
1. Title of the Research	Development of an improved method in BSTI for analysis of Benzoic Acid and Sorbic Acid for fruits and vegetable products.
2. Research Problem	The producers have been using Benzoic Acid and Sorbic Acid as common chemical preservatives in fruits and vegetable products. There are many defined limits of preservatives in product categories in BDS. The existing method of BSTI is time consuming and manually operated. Therefore, it is necessary to develop an improve method to determine Benzoic Acid and Sorbic Acid accurately in the BSTI laboratory through High Performance Liquid Chromatography (HPLC).
3. Justification	The main route of exposure of the general population to benzoic acid and sorbic acid is likely via foodstuffs that contain the substances naturally or added as antimicrobial agents. There are a few analyses of processed foodstuffs available. They refer to different types of food items like fruit juice, fruit drinks, tomato ketchup etc. Food additive exceeding the permitted levels may cause some adverse reactions, including metabolic acidosis, convulsions, asthma, and allergic reactions. Excess amount of additives are introduced to the food, or the wrong additive is introduced through formulation error. In fruit juices carcinogenic compound benzene is produced due to the presence of benzoic acid and ascorbic acid and this is stimulated by the exposure of light and heat. BSTI set the maximum level of Benzoic and Sorbic acid in many fruit and vegetable product like Fruit juice, Fruit drinks, Fruit -Squash, Jam, Jelly, Fruit Syrup, Tomato ketchup, Tomato paste etc. Bangladesh Food Safety Authority (BFSA) also published a regulation regarding use of additives in 2017. Now many company exporting processed fruits and vegetable products, so it will increase the income of the Government of Bangladesh as well as of BSTI. So it is necessary to analyze Benzoic and Sorbic acid accurately.
4. Gap of Previous Research	Presently titrimetric method is using in BSTI for analysis of Benzoic acid. There are many chemical steps involved in this method and sometimes it is difficult to interpret accurate result from titrimetric method.
5. Audience	The Scientist, Laboratory analyst throughout the world will be the audience.
6. Questions	What are the demerits of the existing method? How BSTI can develop a method to be effective, easier, rapid and fit for purpose for analysis of Benzoic and Sorbic acid by using HPLC in BSTI Laboratory for fruits and vegetable products.
7. Purpose	To develop an efficient and improved method for analysis of Benzoic and Sorbic acid by HPLC in BSTI Laboratory for fruits and vegetable products.
8. Title	Development of an improved method in BSTI for analysis of Benzoic Acid and Sorbic Acid for fruits and vegetable products.
9. Methodology	Extraction of benzoic acid and/or sorbic acid from a test portion will be done by using a mixture of ammonium acetate buffer solution and methanol, under acidic condition. The concentration of benzoic and/or sorbic acid will be determined by means of high performance liquid chromatography (HPLC) using a reverse phase

	column and ultraviolet (UV) detector. This validation is to prove that the method developed for the determination of benzoic and/or sorbic acid in fruits and vegetable products is suitable for its intended use "*fit for purpose*". Method Validation will performed using Apple juice as a representative matrix.
10. Time Frame and Tentative Budget	The project needs 06 (Six) months time depending on financial and logistic support. It requires approximately Taka 1,50,000/- (One lac and fifty thousand) for sample collection, procurement of following reagent, chemicals, Certified Reference Materials, Spares of HPLC, sample preparation accessories etc. a) Certified Reference Materials- Tk. 40,000 (Forty Thousand only). b) Reagent and Chemical- Tk. 20,000 (Twenty Thousand only). c) Consumables of HPLC- Tk. 60,000 (Sixty Thousand only). d) Contingency, Travel, Training, Stationary, etc. and others for research work Tk. 30,000 (Thirty Thousand only). This is a tentative budget. Expenditure for each category may increase or decrease at purchase time (with constant total budget).
11. Bibliography	Bibliography will be given at the end of research paper.

Method Validation Protocol

In this research at first the analytical methods were validated. The validation was performed as described in this study in line with international guideline Eurachem**.** After validation of methods the samples were analyzed using that validated method. The following method validation performance characteristics were performed in this study.

(a) Selectivity
(b) Limit of Detection (LOD)
(c) Limit of Quantification(LOQ)
(d) Working Range and Linearity
(e) Accuracy (Recovery)
(f) Precision (Repeatability)

Selectivity

Selectivity relates to the extent to which the method can be used to determine particular analytes in mixtures or matrices without interferences from other components of similar behavior.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ was calculated using the following formula

$$s_0' = s_0 \sqrt{\frac{1}{n} + \frac{1}{n_b}}$$

Where,
S_0 is the estimated standard deviation of single results at or near zero concentration
$S_0'$is the standard deviation used for calculating LOD and LOQ.
n is the number of replicate observations averaged when reporting results where each replicate is obtained following the entire measurement procedure.
n_b is the number of blank observations averaged when calculating the blank correction according to the measurement procedure.

LOD was calculated as 3×So′ and LOQ was calculated as 10×So′

Working Range and Linearity

The working range is an interval, in which a method provides results with an acceptable uncertainty. The lower end of the working range is bounded by the limit of quantification LOQ.

The upper end of the working range is defined by concentrations at which significant anomalies in the analytical sensitivity are observed.

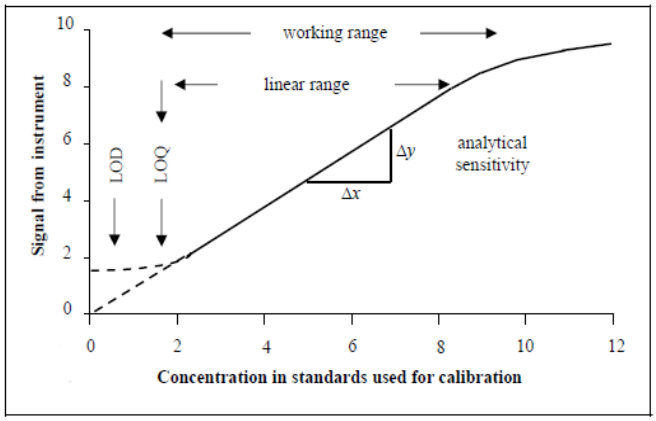


Figure 01: Analytical sensitivity, working range and linear range

The Figure-01 shows a response curve obtained with an instrumental method. The working range, linear range, analytical sensitivity, LOD and LOQ are identified**.**

Accuracy (Recovery)

Accuracy is the closeness of a single result to a reference value. Method validation need to investigate the accuracy of results by considering both systematic and random effects on single results.

Accuracy can be expressed as a relative recovery

$$R(\%) = \frac{x' - \bar{x}}{x_{spike}} \times 100$$

x′ = is the mean value of the spiked sample, $\bar{x}$ is the mean value of unspike sample and x_{spike} is the added concentration.

Precision (Repeatability)

Replication is essential for obtaining reliable estimation of precision. Replicate analysis are designed to take into consideration of all the variations in analytical conditions which is expected during routine use of the method. Precision is expressed as a relative standard deviation since it is approximately constant over the range of interest**.**

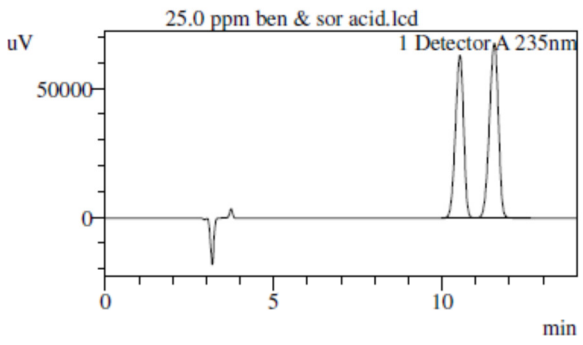
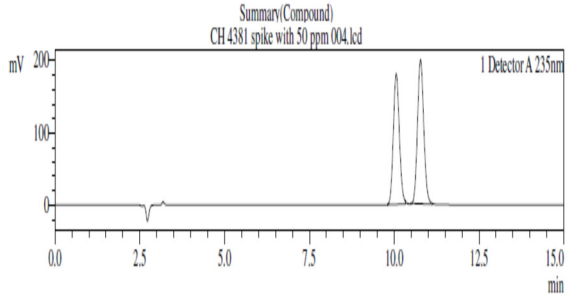
Relative Standard Deviation is calculated as RSD % = $\frac{SD}{Average} \times 100$

Method Validation Report

Method validation report for determination of Benzoic acid and Sorbic acid in Fruit Drink by High Performance Liquid Chromatograph (HPLC)

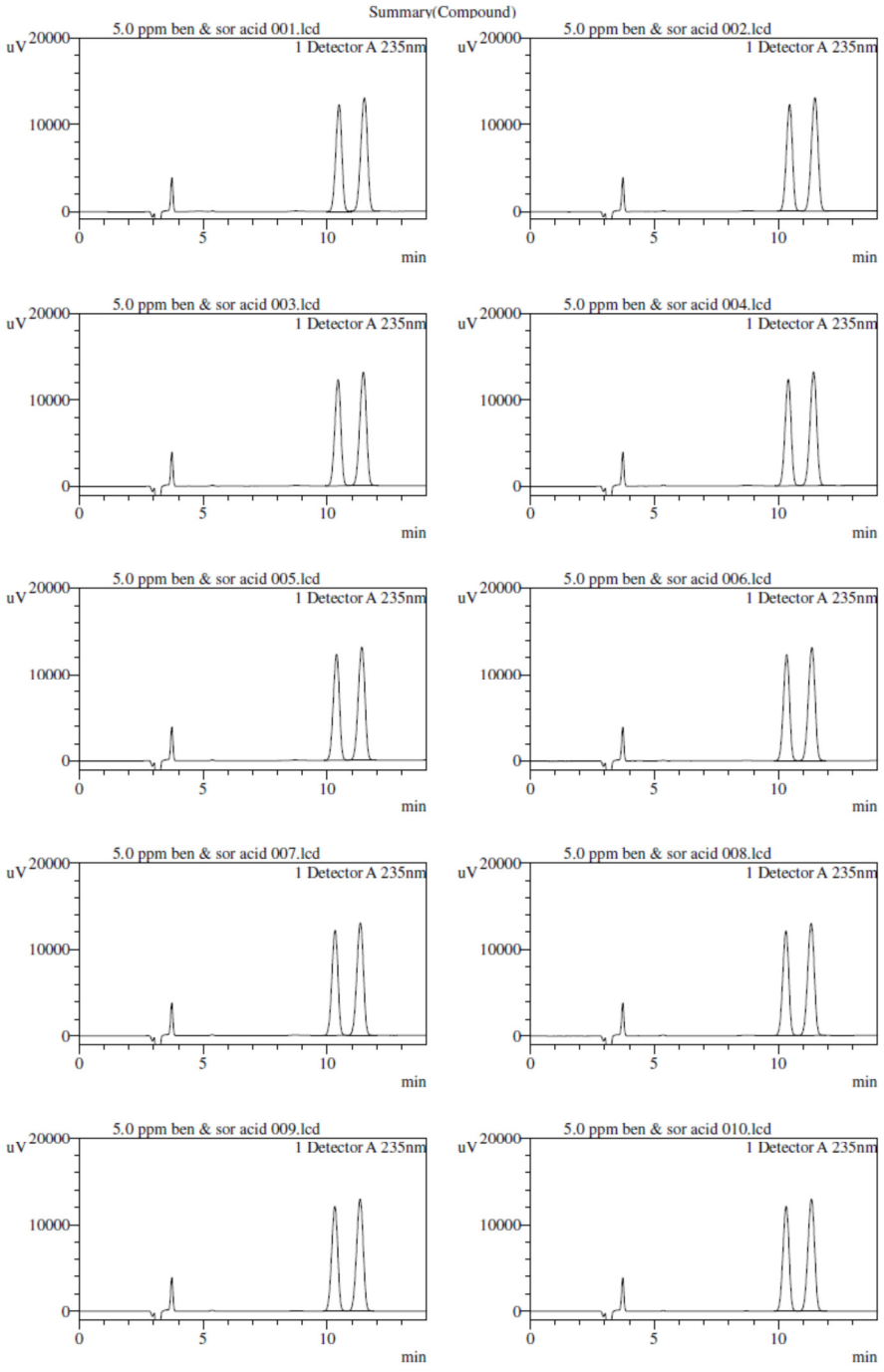
Selectivity

Analytical selectivity relates to "the extent to which the method can be used to determine particular analytes in mixtures or matrices without interferences from other components of similar behavior" A peak in a chromatographic trace identified as being due to the analyte of interest on the basis that an Reference Material (RM) containing the analyte generates a signal at the same point on the chromatogram.

	
Chromatogram of Certified Reference Material(CRM)of Benzoic Acid (RT10.527) and Sorbic Acid(RT11.549) at Concentration 25 mg/L	Chromatogram of Fruit Drinks Sample Spiked with Certified Reference Material of Benzoic Acid (RT10.509) and Sorbic Acid (RT11.449) at Concentration 50 mg/L

The above chromatograms are the supportive evidence to demonstrate selectivity of Benzoic Acid and Sorbic Acid in Fruit Drinks.

Limit of Detection (LOD) and Limit of Quantification (LOQ)



<< Detector A >>
ID#1 Compound Name: Benzoic Acid

Title	Sample Name	Ret. Time	Area
5.0 ppm ben & sor acid 001.lcd	5.0 ppm ben &	10.475	210328
5.0 ppm ben & sor acid 002.lcd	5.0 ppm ben &	10.459	210759
5.0 ppm ben & sor acid 003.lcd	5.0 ppm ben &	10.434	211390
5.0 ppm ben & sor acid 004.lcd	5.0 ppm ben &	10.406	211979
5.0 ppm ben & sor acid 005.lcd	5.0 ppm ben &	10.374	211289
5.0 ppm ben & sor acid 006.lcd	5.0 ppm ben &	10.340	210980
5.0 ppm ben & sor acid 007.lcd	5.0 ppm ben &	10.316	209550
5.0 ppm ben & sor acid 008.lcd	5.0 ppm ben &	10.310	208900
5.0 ppm ben & sor acid 009.lcd	5.0 ppm ben &	10.306	209404
5.0 ppm ben & sor acid 010.lcd	5.0 ppm ben &	10.319	211147

ID#2 Compound Name: Sorbic Acid

Title	Sample Name	Ret. Time	Area
5.0 ppm ben & sor acid 001.lcd	5.0 ppm ben &	11.497	247283
5.0 ppm ben & sor acid 002.lcd	5.0 ppm ben &	11.480	247703
5.0 ppm ben & sor acid 003.lcd	5.0 ppm ben &	11.455	247988
5.0 ppm ben & sor acid 004.lcd	5.0 ppm ben &	11.426	248557
5.0 ppm ben & sor acid 005.lcd	5.0 ppm ben &	11.393	247613
5.0 ppm ben & sor acid 006.lcd	5.0 ppm ben &	11.358	247345
5.0 ppm ben & sor acid 007.lcd	5.0 ppm ben &	11.334	246009
5.0 ppm ben & sor acid 008.lcd	5.0 ppm ben &	11.328	245094
5.0 ppm ben & sor acid 009.lcd	5.0 ppm ben &	11.324	245464
5.0 ppm ben & sor acid 010.lcd	5.0 ppm ben &	11.339	247474

We used the below formula for calculation of LOD and LOQ

$$s_0' = s_0 \sqrt{\frac{1}{m} + \frac{1}{n_b}}$$

s_0 is the estimated standard deviation of m single results at or near zero concentration.

s_0' is the standard deviation used for calculating LOD and LOQ.

n is the number of replicate observations averaged when reporting results where each replicate is obtained following the entire measurement procedure.

n_b is the number of blank observations averaged when calculating the blank correction according to the measurement procedure.

LOD= 3×So´ and LOQ= 10×So´

For Benzoic Acid

Sl. No	Area	Conc(mg/L)	Sample Weight(g)	Conc(mg/kg)
1	210328	5.0447	10.0303	5.0295
2	210759	5.0547	10.1661	4.9721
3	211390	5.0693	10.0262	5.0560
4	211979	5.0829	10.1151	5.0251
5	211289	5.0670	10.2652	4.9360
6	210980	5.0598	10.2659	4.9287
7	209550	5.0267	10.2887	4.8856
8	208900	5.0116	10.2622	4.8836
9	209404	5.0233	10.0959	4.9756
10	211147	5.0637	10.0265	5.0503

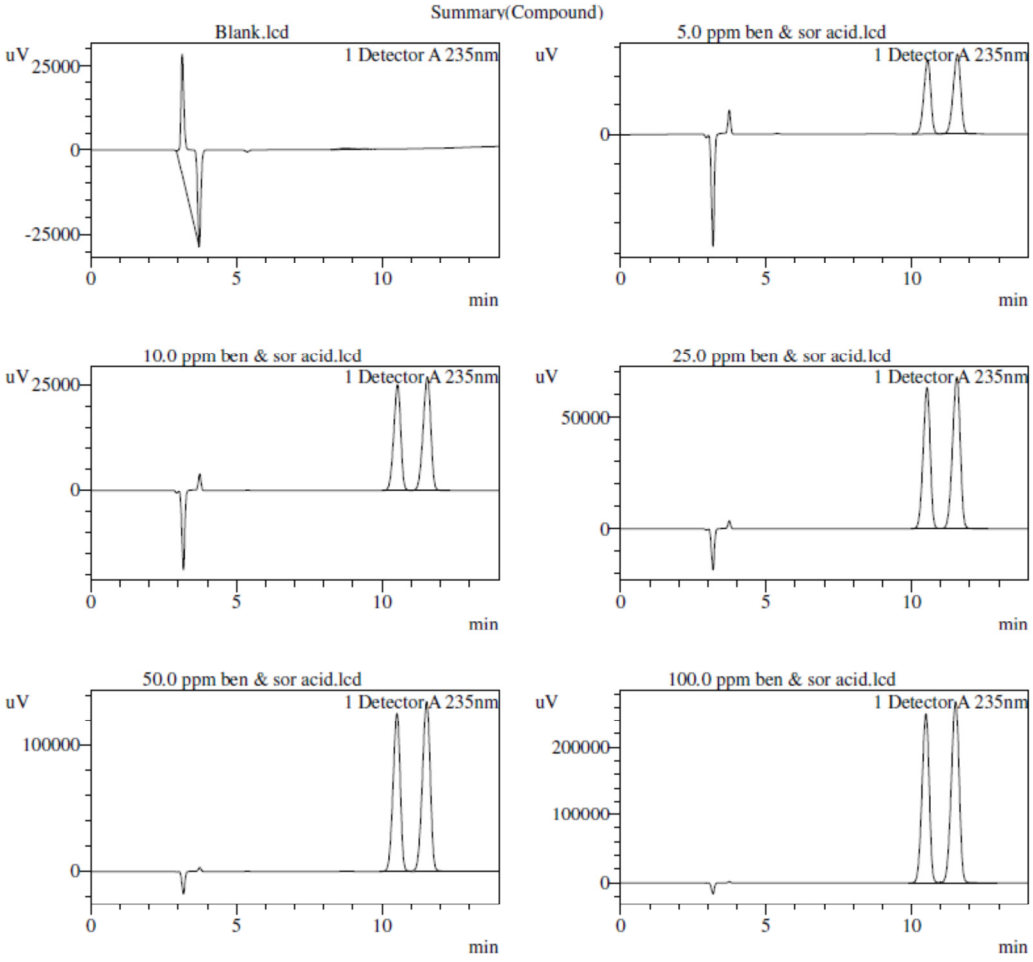
So	1/No. Rep	1/No. Blk	0.10+0.10	SQRT of 0.20	So′	LOD(mg/kg)	LOQ(mg/kg)
0.0647	0.10	0.10	0.20	0.45	0.0290	**0.0869**	**0.2895**

For Sorbic Acid

Sl. No	Area	Conc(mg/L)	Sample Weight(g)	Conc(mg/kg)
1	247283	5.0119	10.0303	4.9967
2	247703	5.0201	10.1661	4.9381
3	247988	5.0257	10.0262	5.0126
4	248557	5.0369	10.1151	4.9796
5	247613	5.0184	10.2652	4.8887
6	247345	5.0131	10.2659	4.8832
7	246009	4.9868	10.2887	4.8469
8	245094	4.9688	10.2622	4.8418
9	245464	4.9761	10.0959	4.9288
10	247474	5.0156	10.0265	5.0024

So	1/No. Rep	1/No. Blk	0.10+0.10	SQRT of 0.20	So′	LOD(mg/kg)	LOQ(mg/kg)
0.0646	0.10	0.10	0.20	0.45	0.0289	**0.0867**	**0.2890**

Working Range and Linearity



<< Detector A >>
ID#1 Compound Name: RT:10.549

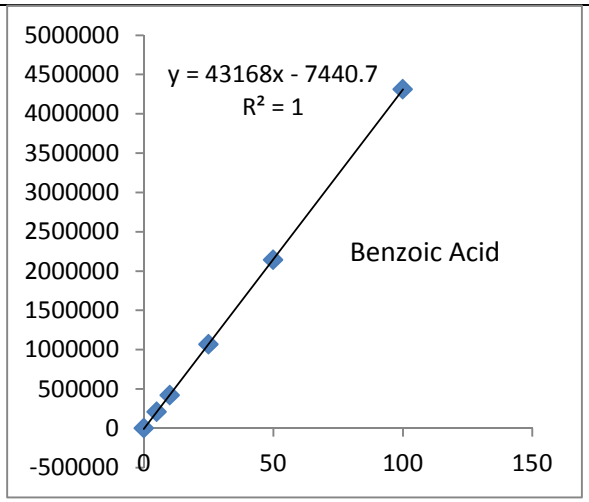
Title	Sample Name	Ret. Time	Area
Blank.lcd	Blank	0.000	0
5.0 ppm ben & sor acid.lcd	5.0 ppm ben &	10.549	209618
10.0 ppm ben & sor acid.lcd	10.0 ppm ben	10.531	421058
25.0 ppm ben & sor acid.lcd	25.0 ppm ben	10.527	1068333
50.0 ppm ben & sor acid.lcd	50.0 ppm ben	10.510	2144616
100.0 ppm ben & sor acid.lcd	100.0 ppm be	10.490	4313631

ID#2 Compound Name: RT:11.570

Title	Sample Name	Ret. Time	Area
Blank.lcd	Blank	0.000	0
5.0 ppm ben & sor acid.lcd	5.0 ppm ben &	11.570	246859
10.0 ppm ben & sor acid.lcd	10.0 ppm ben	11.554	496102
25.0 ppm ben & sor acid.lcd	25.0 ppm ben	11.549	1261947
50.0 ppm ben & sor acid.lcd	50.0 ppm ben	11.531	2528494
100.0 ppm ben & sor acid.lcd	100.0 ppm be	11.508	5077827

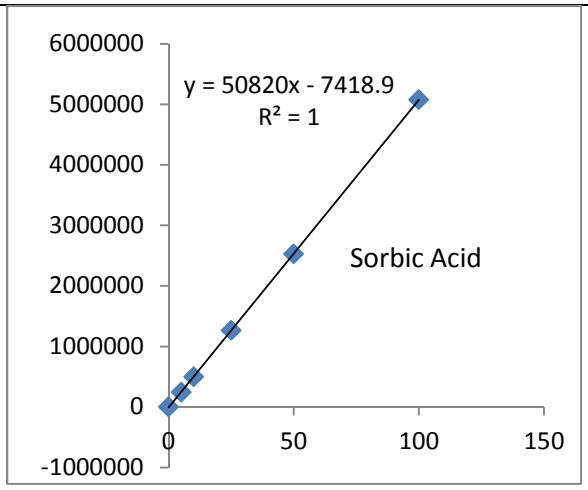
For Benzoic Acid

Conc(mg/L)	Area
0	0
5	209618
10	421058
25	1068333
50	2144616
100	4313631
Intercept	-7440.7385
Slope	43167.8970

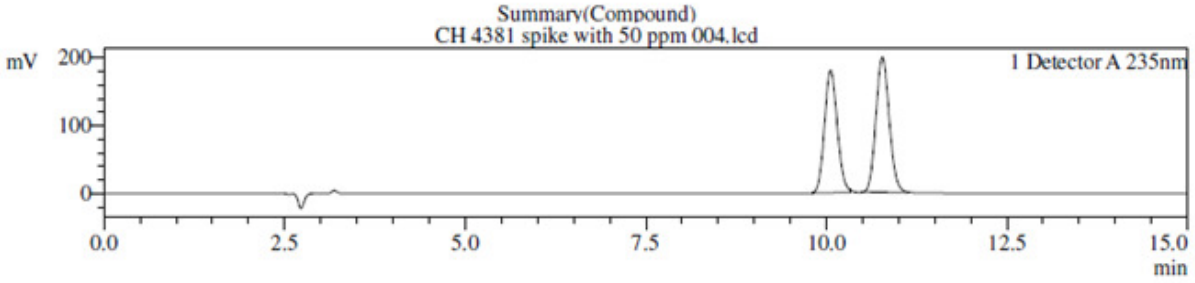


For Sorbic Acid

Conc(mg/L)	Area
0	0
5	246859
10	496102
25	1261947
50	2528494
100	5077827
Intercept	-7418.8906
Slope	50819.6965



Trueness (Spike Recovery)



<< Detector A >>

ID#1 Compound Name: RT:10.059

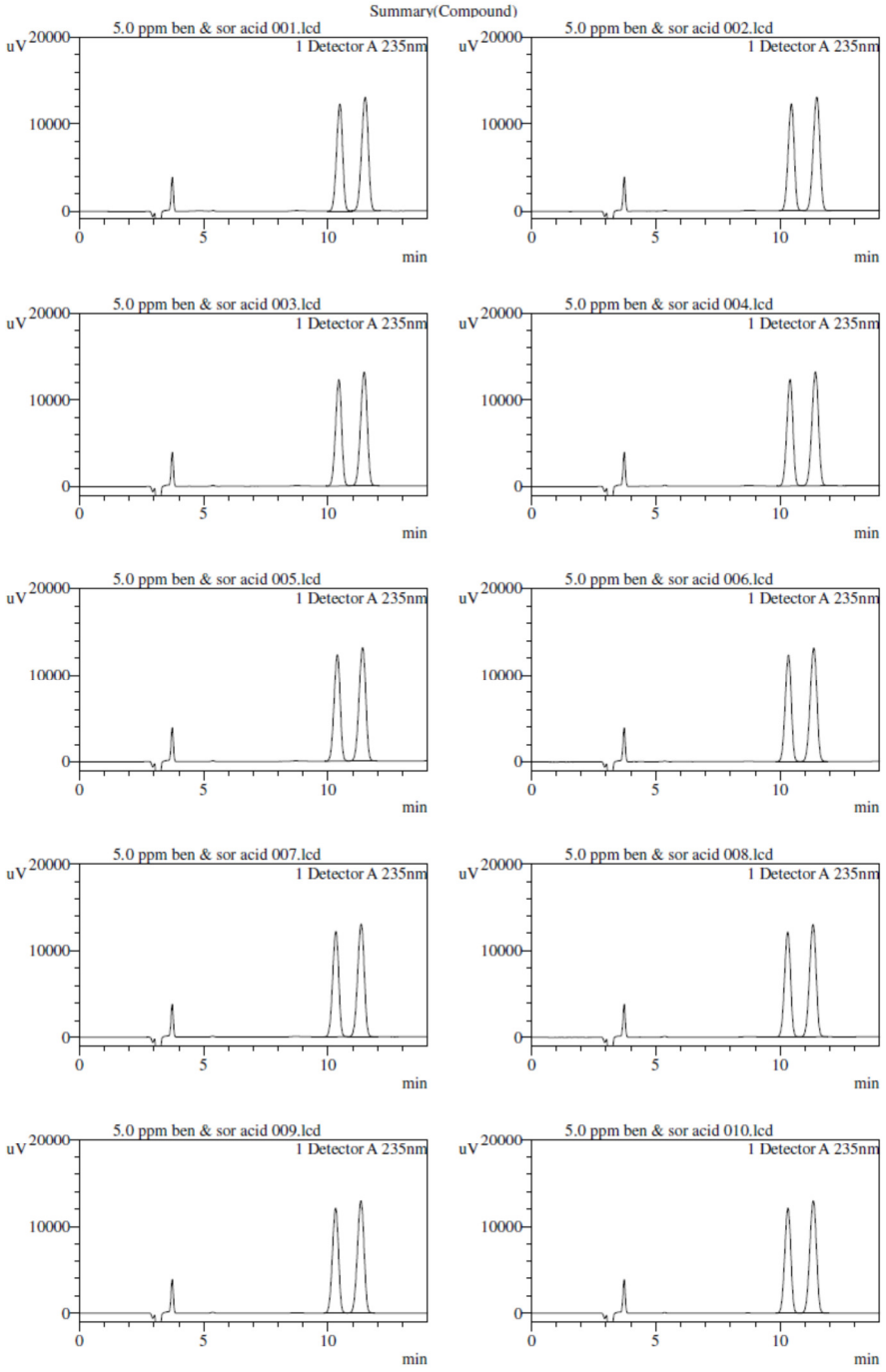
Title	Sample Name	Sample ID	Ret. Time	Area
CH 4381 spike with 50 ppm 004.lcd	CH/4381 spik	CH/4381 spik	10.059	2136429
Standard Deviation			0.000	

ID#2 Compound Name: RT:10.775

Title	Sample Name	Sample ID	Ret. Time	Area
CH 4381 spike with 50 ppm 004.lcd	CH/4381 spik	CH/4381 spik	10.775	2523704
Standard Deviation			0.000	

Name of Analyte	Spike Concentration(mg/L)	Recovery %
Benzoic Acid	50	**99.33**
Sorbic Acid	50	**99.61**

Precision (Repeatability)



<< Detector A >>
ID#1 Compound Name: Benzoic Acid

Title	Sample Name	Ret. Time	Area
5.0 ppm ben & sor acid 001.lcd	5.0 ppm ben &	10.475	210328
5.0 ppm ben & sor acid 002.lcd	5.0 ppm ben &	10.459	210759
5.0 ppm ben & sor acid 003.lcd	5.0 ppm ben &	10.434	211390
5.0 ppm ben & sor acid 004.lcd	5.0 ppm ben &	10.406	211979
5.0 ppm ben & sor acid 005.lcd	5.0 ppm ben &	10.374	211289
5.0 ppm ben & sor acid 006.lcd	5.0 ppm ben &	10.340	210980
5.0 ppm ben & sor acid 007.lcd	5.0 ppm ben &	10.316	209550
5.0 ppm ben & sor acid 008.lcd	5.0 ppm ben &	10.310	208900
5.0 ppm ben & sor acid 009.lcd	5.0 ppm ben &	10.306	209404
5.0 ppm ben & sor acid 010.lcd	5.0 ppm ben &	10.319	211147

ID#2 Compound Name: Sorbic Acid

Title	Sample Name	Ret. Time	Area
5.0 ppm ben & sor acid 001.lcd	5.0 ppm ben &	11.497	247283
5.0 ppm ben & sor acid 002.lcd	5.0 ppm ben &	11.480	247703
5.0 ppm ben & sor acid 003.lcd	5.0 ppm ben &	11.455	247988
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5.0 ppm ben & sor acid 007.lcd	5.0 ppm ben &	11.334	246009
5.0 ppm ben & sor acid 008.lcd	5.0 ppm ben &	11.328	245094
5.0 ppm ben & sor acid 009.lcd	5.0 ppm ben &	11.324	245464
5.0 ppm ben & sor acid 010.lcd	5.0 ppm ben &	11.339	247474

For Benzoic Acid

Sl. No	Area	Conc(mg/L)	Sample Weight(g)	Conc(mg/kg)
1	210328	5.0447	10.0303	5.0295
2	210759	5.0547	10.1661	4.9721
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8	208900	5.0116	10.2622	4.8836
9	209404	5.0233	10.0951	4.9760
10	211147	5.0637	10.0265	5.0503
			STDEV(s)	0.0647
			Average	4.9743
			Precision limit	**0.1813**
			RSD%	**0.1301**

For Sorbic Acid

Sl. No	Area	Conc(mg/L)	Sample Weight(g)	Conc(mg/kg)
1	247283	5.0119	10.0303	4.9967
2	247703	5.0201	10.1661	4.9381
3	247988	5.0257	10.0262	5.0126
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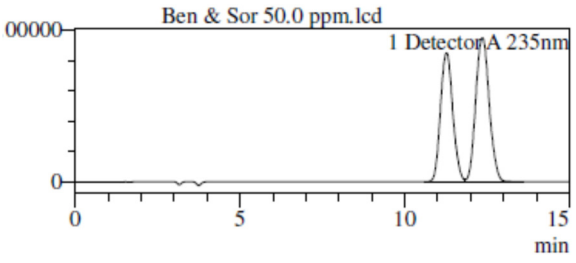
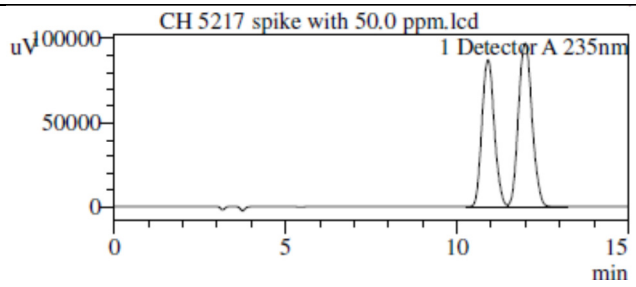
8	245094	4.9688	10.2622	4.8418
9	245464	4.9761	10.0959	4.9288
10	247474	5.0156	10.0265	5.0024
			STDEV(s)	0.0646
			Average	4.9319
			Precision limit	**0.1809**
			RSD%	**0.1310**

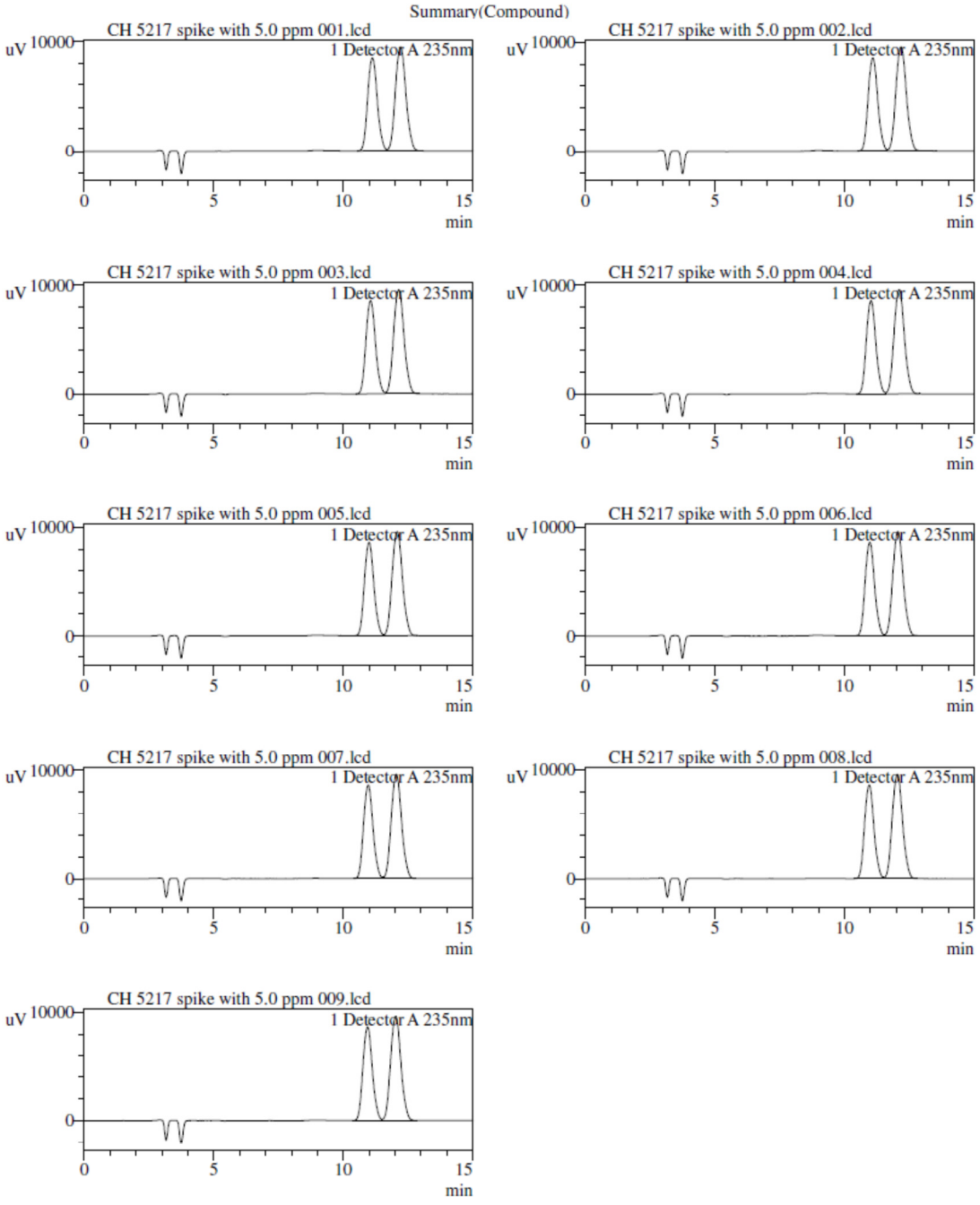
Selectivity

Analytical selectivity relates to "the extent to which the method can be used to determine particular analytes in mixtures or matrices without interferences from other components of similar behavior" A peak in a chromatographic trace identified as being due to the analyte of interest on the basis that an Reference Material (RM) containing the analyte generates a signal at the same point on the chromatogram.

	
Chromatogram of Certified Reference Material(CRM)of Benzoic Acid(RT10.527) and Sorbic Acid(RT11.549) at Concentration 50 mg/L	Chromatogram of Jam Sample Spiked with Certified Reference Material of Benzoic Acid(RT10.509) and Sorbic Acid(RT11.449) at Concentration50 mg/L

The above chromatograms are the supportive evidence to demonstrate selectivity of Benzoic Acid and Sorbic Acid in Jam

Limit of Detection (LOD) and Limit of Quantification (LOQ)



<< Detector A >>

ID#1 Compound Name: RT:11.159

Title	Sample Name	Sample ID	Ret. Time	Area
CH 5217 spike with 5.0 ppm 001.lc	Sample spike	CH/5217 spik	11.125	217848
CH 5217 spike with 5.0 ppm 002.lc	Sample spike	CH/5217 spik	11.097	217790
CH 5217 spike with 5.0 ppm 003.lc	Sample spike	CH/5217 spik	11.056	218016
CH 5217 spike with 5.0 ppm 004.lc	Sample spike	CH/5217 spik	11.025	218058
CH 5217 spike with 5.0 ppm 005.lc	Sample spike	CH/5217 spik	10.998	217867
CH 5217 spike with 5.0 ppm 006.lc	Sample spike	CH/5217 spik	10.978	217839
CH 5217 spike with 5.0 ppm 007.lc	Sample spike	CH/5217 spik	10.964	217962
CH 5217 spike with 5.0 ppm 008.lc	Sample spike	CH/5217 spik	10.955	217744
CH 5217 spike with 5.0 ppm 009.lc	Sample spike	CH/5217 spik	10.940	217908

ID#2 Compound Name: RT:12.249

Title	Sample Name	Sample ID	Ret. Time	Area
CH 5217 spike with 5.0 ppm 001.lc	Sample spike	CH/5217 spik	12.214	267122
CH 5217 spike with 5.0 ppm 002.lc	Sample spike	CH/5217 spik	12.185	266895
CH 5217 spike with 5.0 ppm 003.lc	Sample spike	CH/5217 spik	12.144	267403
CH 5217 spike with 5.0 ppm 004.lc	Sample spike	CH/5217 spik	12.111	267353
CH 5217 spike with 5.0 ppm 005.lc	Sample spike	CH/5217 spik	12.084	267050
CH 5217 spike with 5.0 ppm 006.lc	Sample spike	CH/5217 spik	12.065	267064
CH 5217 spike with 5.0 ppm 007.lc	Sample spike	CH/5217 spik	12.051	267006
CH 5217 spike with 5.0 ppm 008.lc	Sample spike	CH/5217 spik	12.042	266824
CH 5217 spike with 5.0 ppm 009.lc	Sample spike	CH/5217 spik	12.026	267260

We used the below formula for calculation of LOD and LOQ

$$s_0' = s_0 \sqrt{\frac{1}{n} + \frac{1}{n_b}}$$

s_0 is the estimated standard deviation of m single results at or near zero concentration.

s_0' is the standard deviation used for calculating LOD and LOQ.

n is the number of replicate observations averaged when reporting results where each replicate is obtained following the entire measurement procedure.

n_b is the number of blank observations averaged when calculating the blank correction according to the measurement procedure.

LOD= 3×So´ and LOQ= 10×So´

For Benzoic Acid

Sl. No	Area	Conc(mg/L)	Sample Weight(g)	Conc(mg/kg)
1	217848	4.9205	10.0303	4.9056
2	217790	4.9192	10.1661	4.8388
3	218016	4.9244	10.0262	4.9115
4	218058	4.9253	10.1151	4.8693
5	217867	4.9209	10.2652	4.7938
6	217839	4.9203	10.2659	4.7929
7	217962	4.9231	10.2887	4.7850
8	217744	4.9181	10.2622	4.7925
9	217908	4.9219	10.0959	4.8751
10	218114	4.9266	10.0265	4.9136

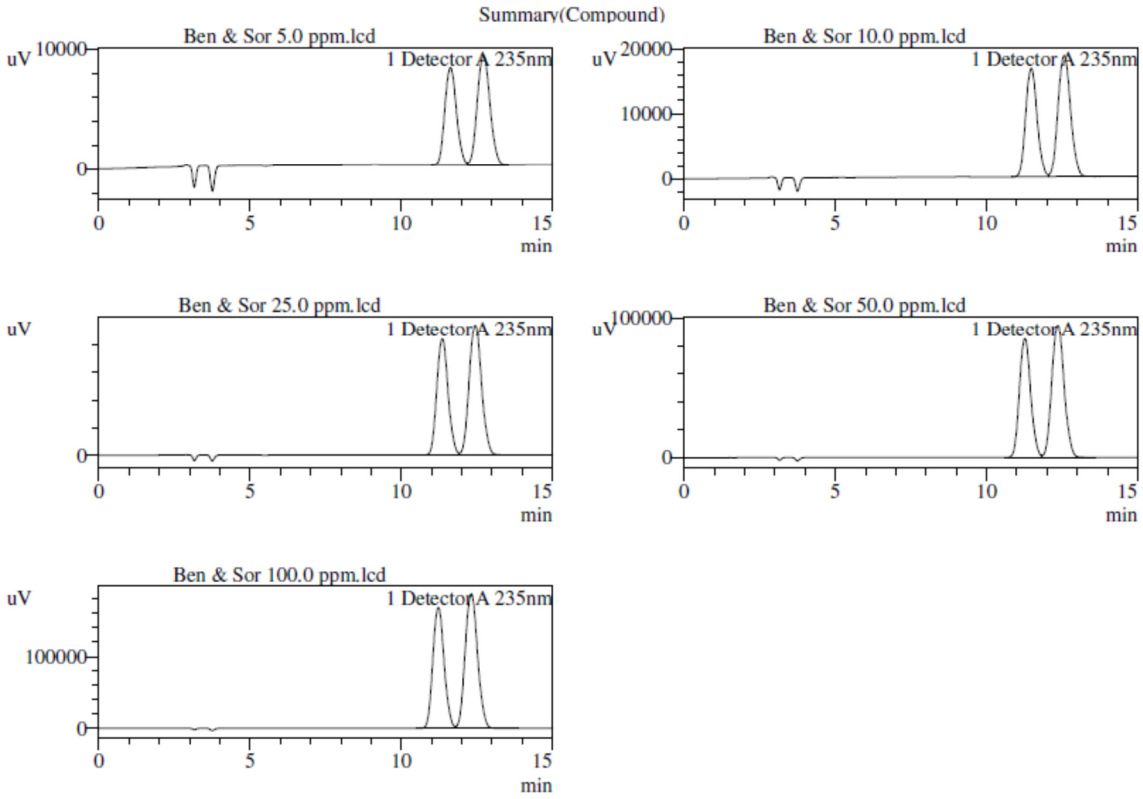
So	1/No. Rep	1/No. Blk	0.1+0.1	SQRT of 0.20	So′	LOD(mg/kg)	LOQ(mg/kg)
0.0537	0.10	0.10	0.20	0.45	0.0240	**0.0721**	**0.2402**

For Sorbic Acid

Sl. No	Area	Conc(mg/L)	Sample Weight(g)	Conc(mg/kg)
1	247283	5.0119	10.0303	4.9967
2	247703	5.0201	10.1661	4.9381
3	247988	5.0257	10.0262	5.0126
4	248557	5.0369	10.1151	4.9796
5	247613	5.0184	10.2652	4.8887
6	247345	5.0131	10.2659	4.8832
7	246009	4.9868	10.2887	4.8469
8	245094	4.9688	10.2622	4.8418
9	245464	4.9761	10.0959	4.9288
10	247474	5.0156	10.0265	5.0024

So	1/No. Rep	1/No. Blk	0.1+0.1	SQRT of 0.20	So′	LOD(mg/kg)	LOQ(mg/kg)
0.0540	0.10	0.10	0.20	0.45	0.0241	**0.0724**	**0.2414**

Working Range and Linearity



<< Detector A >>

ID#1 Compound Name: RT:11.630

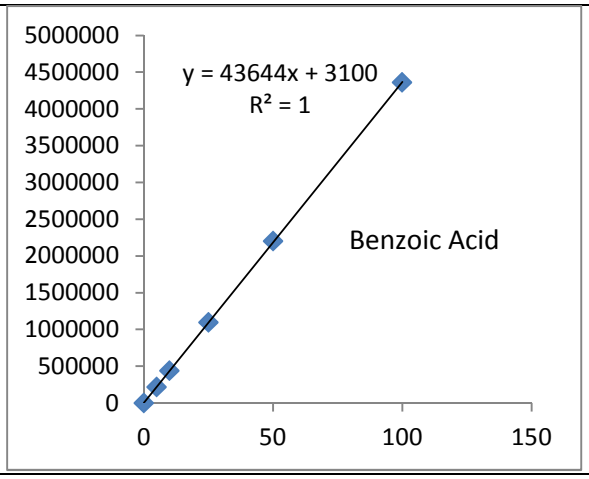
Title	Sample Name	Sample ID	Ret. Time	Area
Ben & Sor 5.0 ppm.lcd	Standard-1	Ben & Sor 5.0	11.630	217223
Ben & Sor 10.0 ppm.lcd	Standard-2	Ben & Sor 10.	11.483	439612
Ben & Sor 25.0 ppm.lcd	Standard-3	Ben & Sor 25.	11.359	1093594
Ben & Sor 50.0 ppm.lcd	Standard-4	Ben & Sor 50.	11.273	2200006
Ben & Sor 100.0 ppm.lcd	Standard-5	Ben & Sor 10(	11.229	4360430

ID#2 Compound Name: RT:12.721

Title	Sample Name	Sample ID	Ret. Time	Area
Ben & Sor 5.0 ppm.lcd	Standard-1	Ben & Sor 5.0	12.721	267176
Ben & Sor 10.0 ppm.lcd	Standard-2	Ben & Sor 10.	12.572	539351
Ben & Sor 25.0 ppm.lcd	Standard-3	Ben & Sor 25.	12.448	1345530
Ben & Sor 50.0 ppm.lcd	Standard-4	Ben & Sor 50.	12.362	2702412
Ben & Sor 100.0 ppm.lcd	Standard-5	Ben & Sor 10(	12.317	5346147

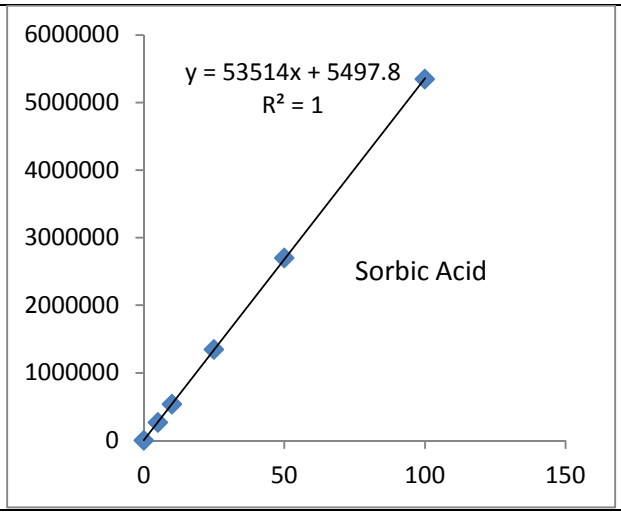
Benzoic Acid

Conc(mg/L)	Area
0	0
5	217223
10	439612
25	1093594
50	2200006
100	4360430
Intercept	3099.9562
Slope	43643.5014

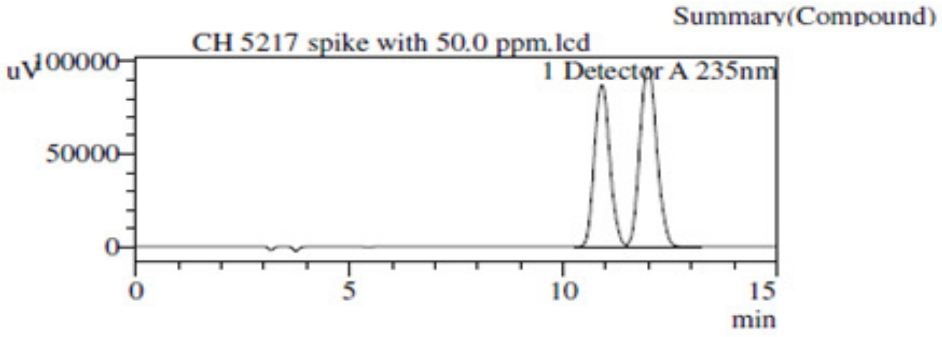


Sorbic Acid

Conc(mg/L)	Area
0	0
5	267176
10	539351
25	1345530
50	2702412
100	5346147
Intercept	5497.7742
Slope	53513.8387



Trueness (Spike Recovery)



<< Detector A >>

ID#1 Compound Name: RT:10.910

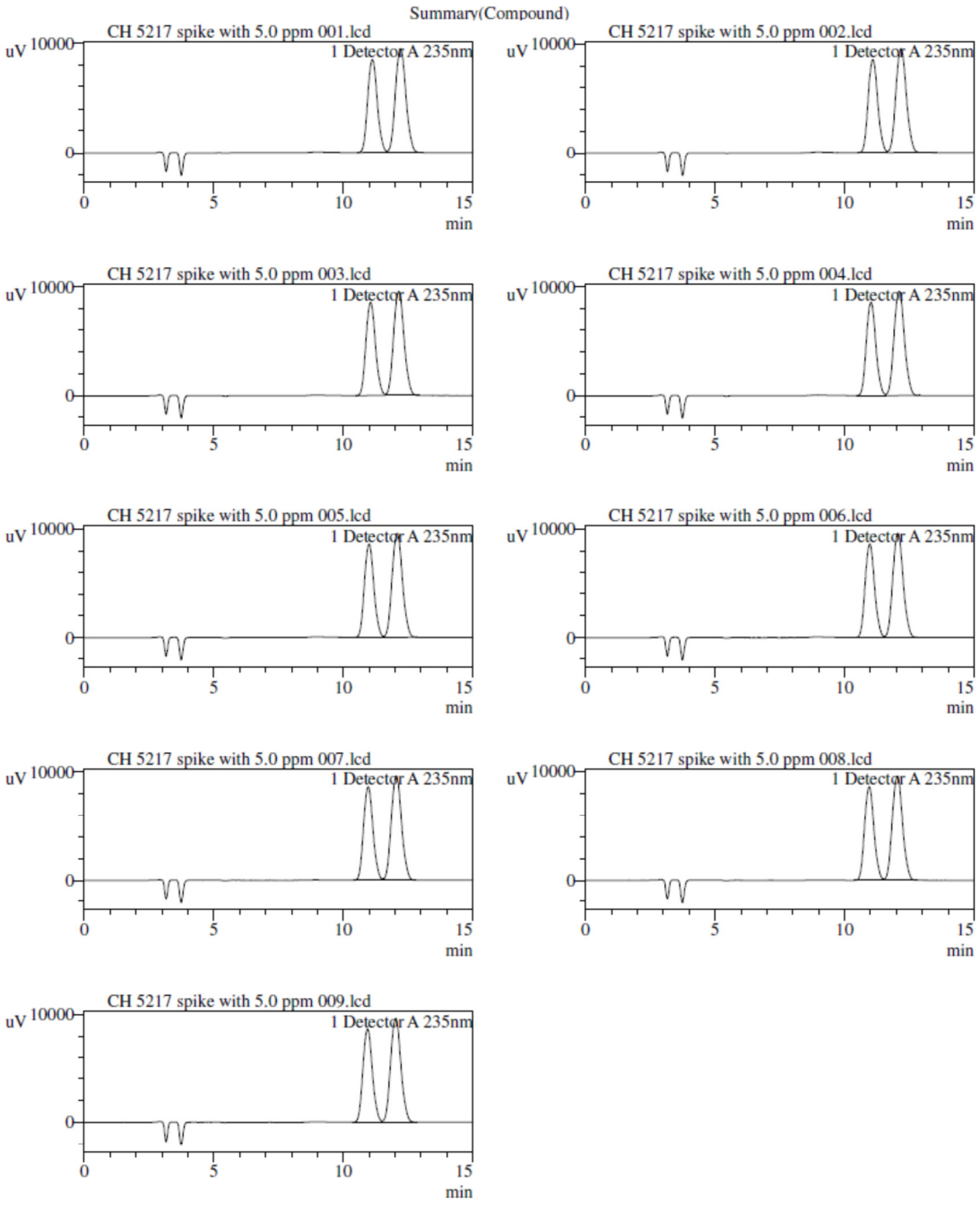
Title	Sample Name	Sample ID	Ret. Time	Area
CH 5217 spike with 50.0 ppm.lcd	Sample spike	CH/5217 spik	10.910	2161250

ID#2 Compound Name: RT:11.994

Title	Sample Name	Sample ID	Ret. Time	Area
CH 5217 spike with 50.0 ppm.lcd	Sample spike	CH/5217 spik	11.994	2658574

Name of Analyte	Spike Concentration(mg/L)	Recovery %
Benzoic Acid	50	**98.90**
Sorbic Acid	50	**99.15**

Precision (Repeatability)



<< Detector A >>
ID#1 Compound Name: RT:11.159

Title	Sample Name	Sample ID	Ret. Time	Area
CH 5217 spike with 5.0 ppm 001.lc	Sample spike	CH/5217 spik	11.125	217848
CH 5217 spike with 5.0 ppm 002.lc	Sample spike	CH/5217 spik	11.097	217790
CH 5217 spike with 5.0 ppm 003.lc	Sample spike	CH/5217 spik	11.056	218016
CH 5217 spike with 5.0 ppm 004.lc	Sample spike	CH/5217 spik	11.025	218058
CH 5217 spike with 5.0 ppm 005.lc	Sample spike	CH/5217 spik	10.998	217867
CH 5217 spike with 5.0 ppm 006.lc	Sample spike	CH/5217 spik	10.978	217839
CH 5217 spike with 5.0 ppm 007.lc	Sample spike	CH/5217 spik	10.964	217962
CH 5217 spike with 5.0 ppm 008.lc	Sample spike	CH/5217 spik	10.955	217744
CH 5217 spike with 5.0 ppm 009.lc	Sample spike	CH/5217 spik	10.940	217908

ID#2 Compound Name: RT:12.249

Title	Sample Name	Sample ID	Ret. Time	Area
CH 5217 spike with 5.0 ppm 001.lc	Sample spike	CH/5217 spik	12.214	267122
CH 5217 spike with 5.0 ppm 002.lc	Sample spike	CH/5217 spik	12.185	266895
CH 5217 spike with 5.0 ppm 003.lc	Sample spike	CH/5217 spik	12.144	267403
CH 5217 spike with 5.0 ppm 004.lc	Sample spike	CH/5217 spik	12.111	267353
CH 5217 spike with 5.0 ppm 005.lc	Sample spike	CH/5217 spik	12.084	267050
CH 5217 spike with 5.0 ppm 006.lc	Sample spike	CH/5217 spik	12.065	267064
CH 5217 spike with 5.0 ppm 007.lc	Sample spike	CH/5217 spik	12.051	267006
CH 5217 spike with 5.0 ppm 008.lc	Sample spike	CH/5217 spik	12.042	266824
CH 5217 spike with 5.0 ppm 009.lc	Sample spike	CH/5217 spik	12.026	267260

For Benzoic Acid

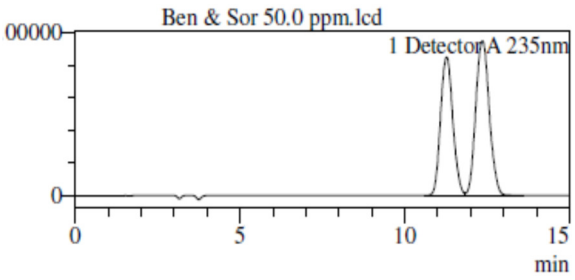
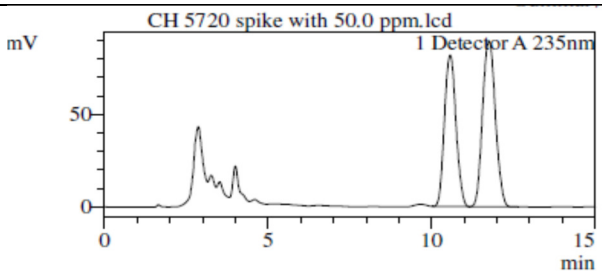
Sl. No	Area	Conc(mg/L)	Sample Weight(g)	Conc(mg/kg)
1	217848	4.9205	10.0303	4.9056
2	217790	4.9192	10.1661	4.8388
3	218021	4.9245	10.0262	4.9116
4	218058	4.9253	10.1151	4.8693
5	217867	4.9209	10.2652	4.7938
6	217839	4.9203	10.2659	4.7929
7	217962	4.9231	10.2887	4.7850
8	217744	4.9181	10.2622	4.7925
9	217908	4.9219	10.0951	4.8755
10	218114	4.9266	10.0265	4.9136
			STDEV(s)	0.0537
			Average	4.8479
			Precision limit	**0.1505**
			RSD%	**0.1109**

For Sorbic Acid

Sl. No	Area	Conc(mg/L)	Sample Weight(g)	Conc(mg/kg)
1	267122	4.8889	10.0303	4.8741
2	266895	4.8847	10.1661	4.8049
3	267403	4.8942	10.0262	4.8814
4	267353	4.8932	10.1151	4.8375
5	267050	4.8876	10.2652	4.7613
6	267064	4.8878	10.2659	4.7612
7	267006	4.8867	10.2887	4.7496
8	266824	4.8833	10.2622	4.7586
9	267260	4.8915	10.0959	4.8450
10	267058	4.8877	10.0265	4.8748
			STDEV(s)	0.0540
			Average	4.8148
			Precision limit	**0.1511**
			RSD%	**0.1121**

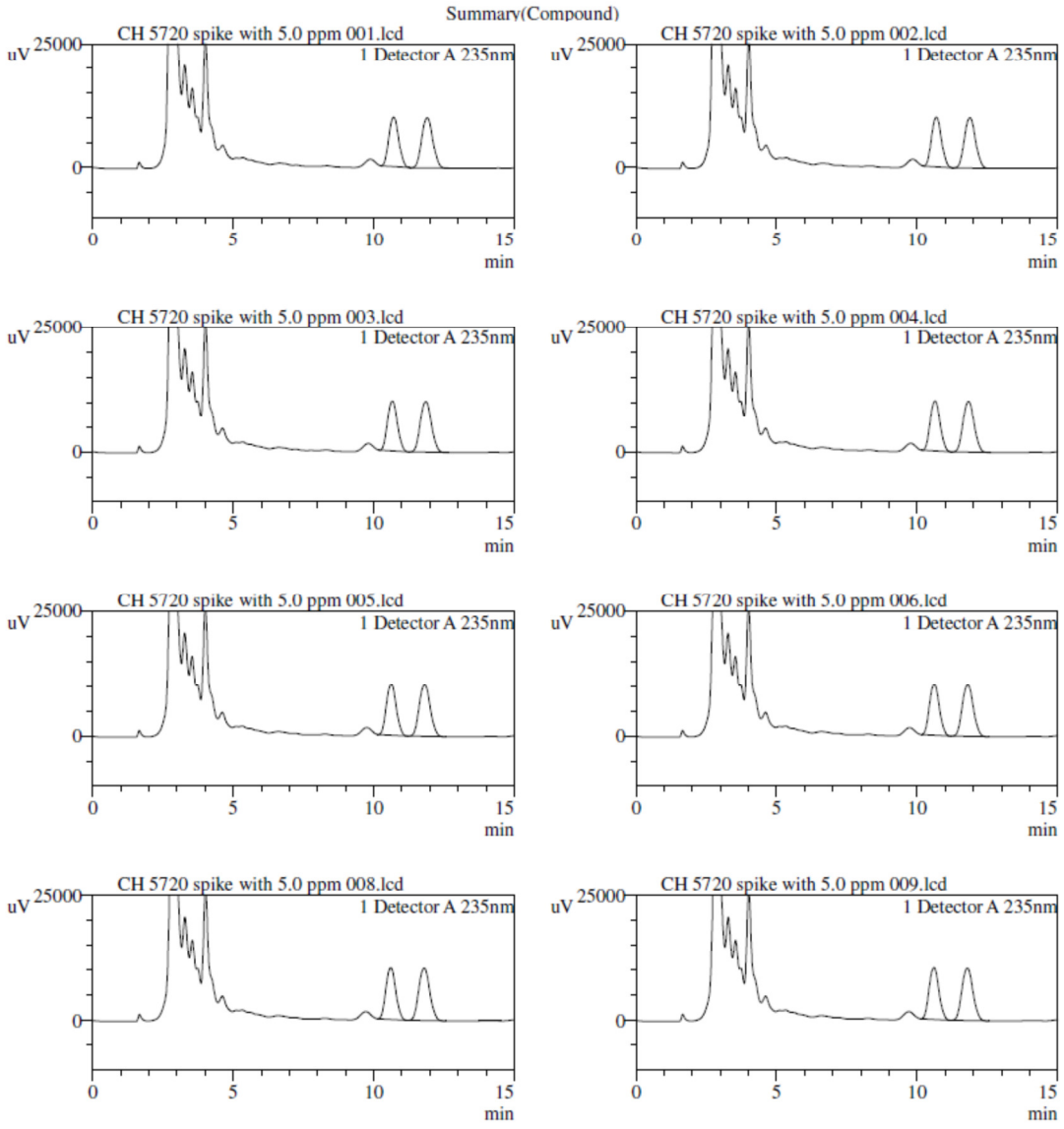
Selectivity

Analytical selectivity relates to "the extent to which the method can be used to determine particular analytes in mixtures or matrices without interferences from other components of similar behavior" A peak in a chromatographic trace identified as being due to the analyte of interest on the basis that an Reference Material (RM) containing the analyte generates a signal at the same point on the chromatogram.

	
Chromatogram of Certified Reference Material(CRM) of Benzoic Acid(RT10.527) and Sorbic Acid(RT11.549) at Concentration 50 mg/L	Chromatogram of Tomato Paste Sample Spiked with Certified Reference Material of Benzoic Acid(RT10.509) and Sorbic Acid(RT11.449) at Concentration 50 mg/L

The above chromatograms are the supportive evidence to demonstrate selectivity of Benzoic Acid and Sorbic Acid in Tomato Paste

Limit of Detection (LOD) and Limit of Quantification (LOQ)



<< Detector A >>
ID#1 Compound Name: RT:10.573

Title	Sample Name	Sample ID	Ret. Time	Area
CH 5720 spike with 5.0 ppm 001.lc	Sample spike	CH/5217 spik	10.710	247371
CH 5720 spike with 5.0 ppm 002.lc	Sample spike	CH/5217 spik	10.687	248011
CH 5720 spike with 5.0 ppm 003.lc	Sample spike	CH/5217 spik	10.663	248532
CH 5720 spike with 5.0 ppm 004.lc	Sample spike	CH/5217 spik	10.639	248865
CH 5720 spike with 5.0 ppm 005.lc	Sample spike	CH/5217 spik	10.624	249367
CH 5720 spike with 5.0 ppm 006.lc	Sample spike	CH/5217 spik	10.613	249140
CH 5720 spike with 5.0 ppm 008.lc	Sample spike	CH/5217 spik	10.607	249395
CH 5720 spike with 5.0 ppm 009.lc	Sample spike	CH/5217 spik	10.600	249363

ID#2 Compound Name: RT:11.760

Title	Sample Name	Sample ID	Ret. Time	Area
CH 5720 spike with 5.0 ppm 001.lc	Sample spike	CH/5217 spik	11.905	284325
CH 5720 spike with 5.0 ppm 002.lc	Sample spike	CH/5217 spik	11.879	284233
CH 5720 spike with 5.0 ppm 003.lc	Sample spike	CH/5217 spik	11.855	284231
CH 5720 spike with 5.0 ppm 004.lc	Sample spike	CH/5217 spik	11.831	284041
CH 5720 spike with 5.0 ppm 005.lc	Sample spike	CH/5217 spik	11.815	284442
CH 5720 spike with 5.0 ppm 006.lc	Sample spike	CH/5217 spik	11.804	284111
CH 5720 spike with 5.0 ppm 008.lc	Sample spike	CH/5217 spik	11.797	283996
CH 5720 spike with 5.0 ppm 009.lc	Sample spike	CH/5217 spik	11.791	283852

We used the below formula for calculation of LOD and LOQ

$$s_0' = s_0 \sqrt{\frac{1}{n} + \frac{1}{n_b}}$$

s_0 is the estimated standard deviation of m single results at or near zero concentration.

s_0' is the standard deviation used for calculating LOD and LOQ.

n is the number of replicate observations averaged when reporting results where each replicate is obtained following the entire measurement procedure.

n_b is the number of blank observations averaged when calculating the blank correction according to the measurement procedure.

LOD = 3×So´ and LOQ = 10×So´

For Benzoic Acid

Sl. No	Area	Conc(mg/L)	Sample Weight(g)	Conc(mg/kg)
1	247371	5.5970	10.0303	5.5801
2	248011	5.6116	10.1661	5.5199
3	248532	5.6236	10.0262	5.6089
4	248865	5.6312	10.1151	5.5671
5	249367	5.6427	10.2652	5.4969
6	249140	5.6375	10.2659	5.4915
7	249395	5.6433	10.2887	5.4850
8	249363	5.6426	10.2622	5.4984
9	247908	5.6093	10.0959	5.5560
10	248114	5.6140	10.0265	5.5991

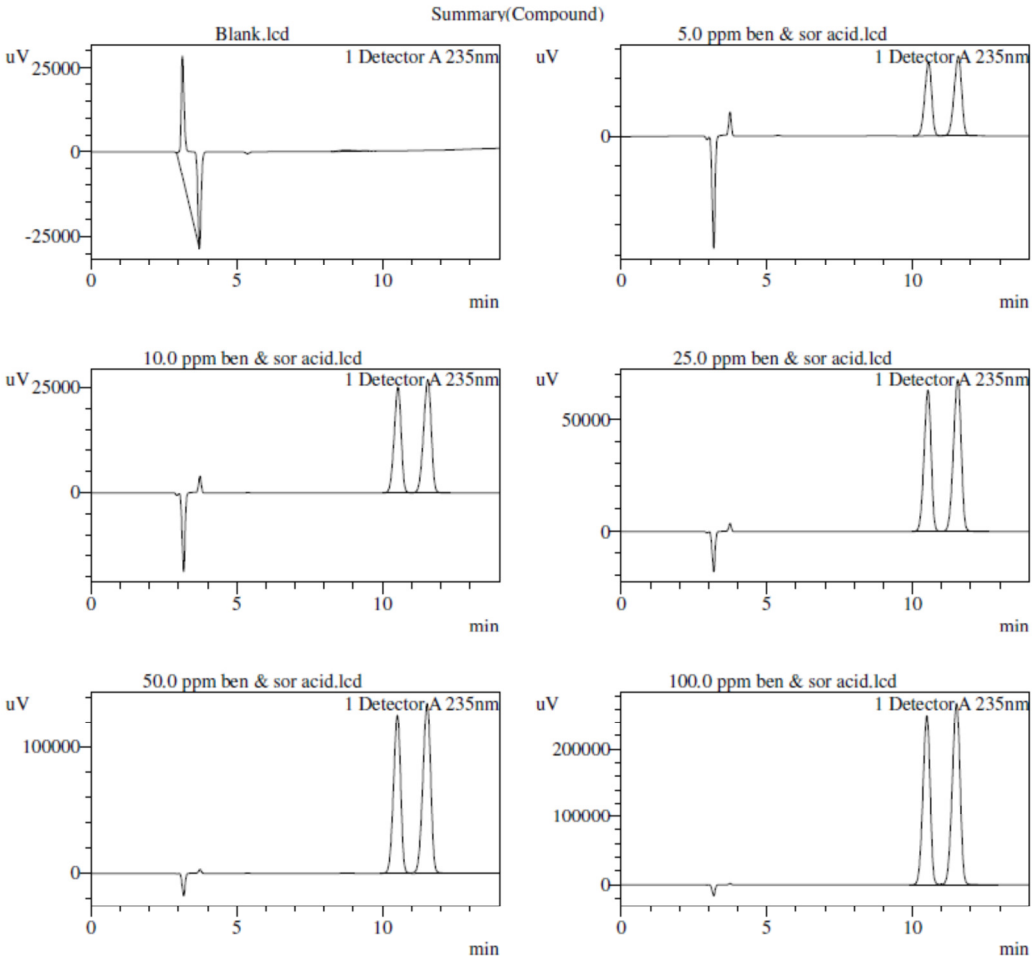
So	1/No. Rep	1/No. Blk	G20+H20	SQRT of 0.20	So′	LOD(mg/kg)	LOQ(mg/kg)
0.0474	0.10	0.10	0.20	0.45	0.0212	**0.0636**	**0.2119**

For Sorbic Acid

Sl. No	Area	Conc(mg/L)	Sample Weight(g)	Conc(mg/kg)
1	284325	5.2104	10.0303	5.1946
2	284233	5.2087	10.1661	5.1236
3	284231	5.2086	10.0262	5.1950
4	284041	5.2051	10.1151	5.1458
5	284442	5.2126	10.2652	5.0779
6	284111	5.2064	10.2659	5.0715
7	283996	5.2042	10.2887	5.0582
8	283852	5.2015	10.2622	5.0686
9	287260	5.2652	10.0959	5.2152
10	287058	5.2614	10.0265	5.2475

So	1/No. Rep	1/No. Blk	G20+H20	SQRT of 0.20	So′	LOD(mg/kg)	LOQ(mg/kg)
0.0698	0.10	0.10	0.20	0.45	0.0312	**0.0937**	**0.3122**

Working Range and Linearity



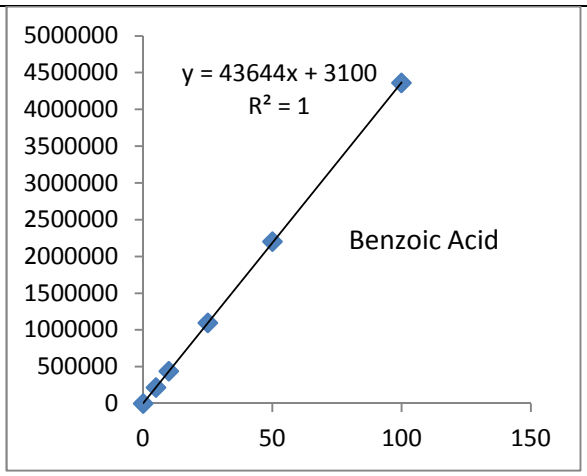
<< Detector A >>
ID#1 Compound Name: RT:10.549

Title	Sample Name	Ret. Time	Area
Blank.lcd	Blank	0.000	0
5.0 ppm ben & sor acid.lcd	5.0 ppm ben &	10.549	209618
10.0 ppm ben & sor acid.lcd	10.0 ppm ben	10.531	421058
25.0 ppm ben & sor acid.lcd	25.0 ppm ben	10.527	1068333
50.0 ppm ben & sor acid.lcd	50.0 ppm ben	10.510	2144616
100.0 ppm ben & sor acid.lcd	100.0 ppm ben	10.490	4313631

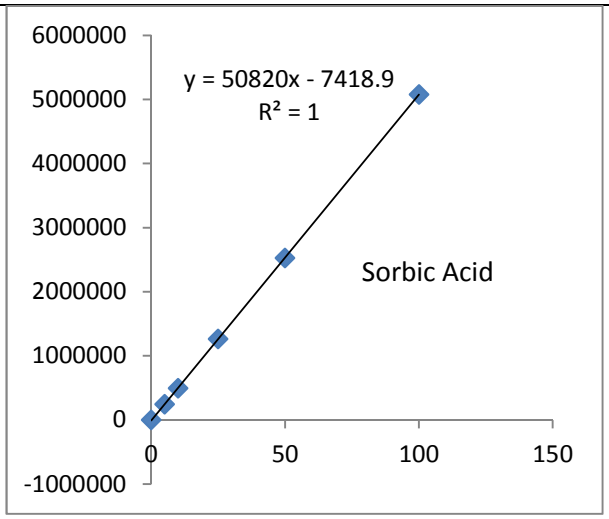
ID#2 Compound Name: RT:11.570

Title	Sample Name	Ret. Time	Area
Blank.lcd	Blank	0.000	0
5.0 ppm ben & sor acid.lcd	5.0 ppm ben &	11.570	246859
10.0 ppm ben & sor acid.lcd	10.0 ppm ben	11.554	496102
25.0 ppm ben & sor acid.lcd	25.0 ppm ben	11.549	1261947
50.0 ppm ben & sor acid.lcd	50.0 ppm ben	11.531	2528494
100.0 ppm ben & sor acid.lcd	100.0 ppm ben	11.508	5077827

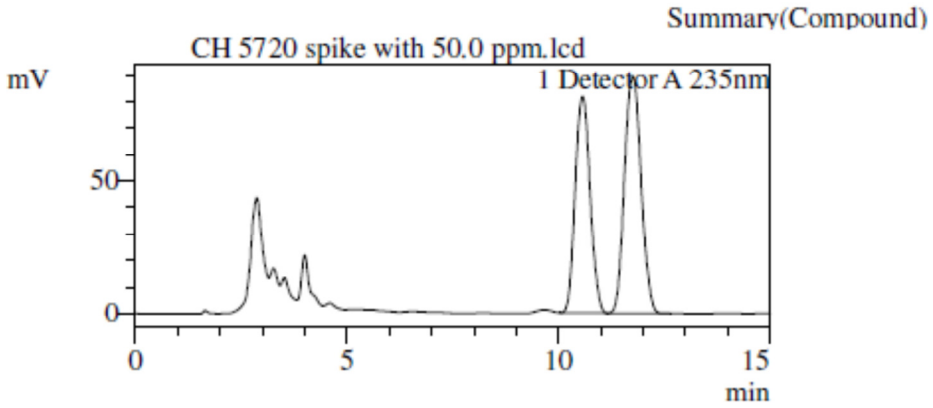
Conc(mg/L)	Area
0	0
5	217223
10	439612
25	1093594
50	2200006
100	4360430
Intercept	3099.9562
Slope	43643.5014



Conc(mg/L)	Area
0	0
5	246859
10	496102
25	1261947
50	2528494
100	5077827
Intercept	-7418.8906
Slope	50819.6965



Trueness (Spike Recovery)



<< Detector A >>

ID#1 Compound Name: RT:10.573

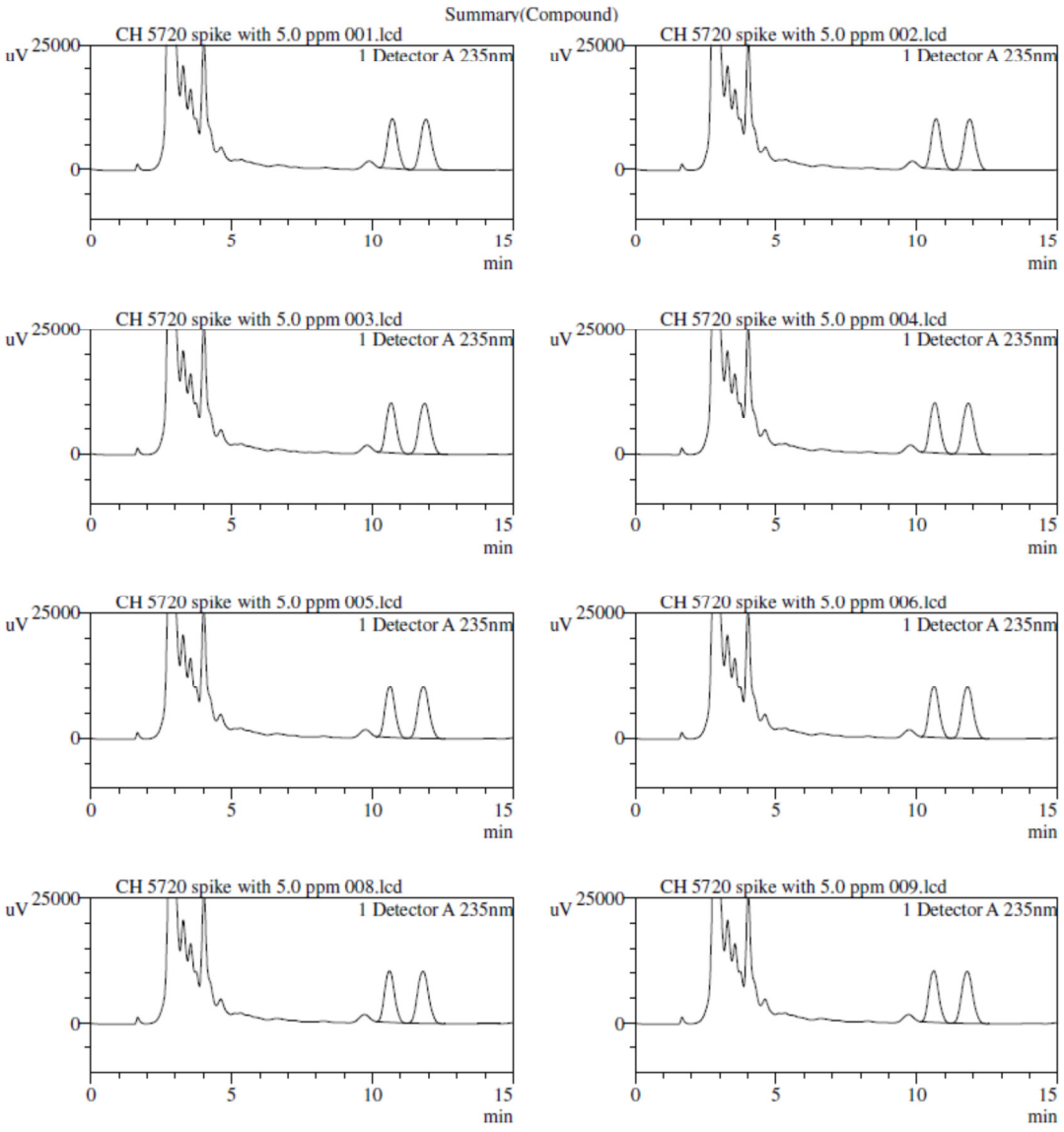
Title	Sample Name	Sample ID	Ret. Time	Area
CH 5720 spike with 50.0 ppm.lcd	Sample spike	CH/5217 spik	10.573	2004172

ID#2 Compound Name: RT:11.760

Title	Sample Name	Sample ID	Ret. Time	Area
CH 5720 spike with 50.0 ppm.lcd	Sample spike	CH/5217 spik	11.760	2448812

Name of Analyte	Spike Concentration(mg/L)	Recovery %
Benzoic Acid	50	**91.70**
Sorbic Acid	50	**91.31**

Precision (Repeatability)



<< Detector A >>

ID#1 Compound Name: RT:10.573

Title	Sample Name	Sample ID	Ret. Time	Area
CH 5720 spike with 5.0 ppm 001.lc	Sample spike	CH/5217 spik	10.710	247371
CH 5720 spike with 5.0 ppm 002.lc	Sample spike	CH/5217 spik	10.687	248011
CH 5720 spike with 5.0 ppm 003.lc	Sample spike	CH/5217 spik	10.663	248532
CH 5720 spike with 5.0 ppm 004.lc	Sample spike	CH/5217 spik	10.639	248865
CH 5720 spike with 5.0 ppm 005.lc	Sample spike	CH/5217 spik	10.624	249367
CH 5720 spike with 5.0 ppm 006.lc	Sample spike	CH/5217 spik	10.613	249140
CH 5720 spike with 5.0 ppm 008.lc	Sample spike	CH/5217 spik	10.607	249395
CH 5720 spike with 5.0 ppm 009.lc	Sample spike	CH/5217 spik	10.600	249363

ID#2 Compound Name: RT:11.760

Title	Sample Name	Sample ID	Ret. Time	Area
CH 5720 spike with 5.0 ppm 001.lc	Sample spike	CH/5217 spik	11.905	284325
CH 5720 spike with 5.0 ppm 002.lc	Sample spike	CH/5217 spik	11.879	284233
CH 5720 spike with 5.0 ppm 003.lc	Sample spike	CH/5217 spik	11.855	284231
CH 5720 spike with 5.0 ppm 004.lc	Sample spike	CH/5217 spik	11.831	284041
CH 5720 spike with 5.0 ppm 005.lc	Sample spike	CH/5217 spik	11.815	284442
CH 5720 spike with 5.0 ppm 006.lc	Sample spike	CH/5217 spik	11.804	284111
CH 5720 spike with 5.0 ppm 008.lc	Sample spike	CH/5217 spik	11.797	283996
CH 5720 spike with 5.0 ppm 009.lc	Sample spike	CH/5217 spik	11.791	283852

For Benzoic Acid

Sl. No	Area	Conc(mg/L)	Sample Weight(g)	Conc(mg/kg)
1	247371	5.5970	10.0303	5.5801
2	248011	5.6116	10.1661	5.5199
3	248532	5.6236	10.0262	5.6089
4	248865	5.6312	10.1151	5.5671
5	249367	5.6427	10.2652	5.4969
6	249140	5.6375	10.2659	5.4915
7	249395	5.6433	10.2887	5.4850
8	249363	5.6426	10.2622	5.4984
9	247908	5.6093	10.0951	5.5564
10	248114	5.6140	10.0265	5.5991
			STDEV(s)	0.0474
			Average	5.5403
			Precision limit	**0.1327**
			RSD%	**0.0855**

For Sorbic Acid

Sl. No	Area	Conc(mg/L)	Sample Weight(g)	Conc(mg/kg)
1	284325	5.2104	10.0303	5.1946
2	284233	5.2087	10.1661	5.1236
3	284231	5.2086	10.0262	5.1950
4	284041	5.2051	10.1151	5.1458
5	284442	5.2126	10.2652	5.0779
6	284111	5.2064	10.2659	5.0715
7	283996	5.2042	10.2887	5.0582
8	283852	5.2015	10.2622	5.0686
9	287260	5.2652	10.0959	5.2152
10	287058	5.2614	10.0265	5.2475
			STDEV(s)	0.0698
			Average	5.1398
			Precision limit	**0.1955**
			RSD%	**0.1358**

Discussion and Conclusion

In this newly developed improved method for analysis of Benzoic Acid and Sorbic Acid in fruits and vegetable products, High Performance Liquid Chromatograph (HPLC) was used. The fruit drink, jam and tomato paste were used as representative matrix for method validation. International guide line Eurachem was used as method validation protocol. All method validation performance characteristics: Selectivity, Limit of Detection (LOD), Limit of Quantification (LOQ), Working Range and Linearity, Accuracy (Recovery) and Precision (Repeatability) were fulfilled for these three matrix. The method is sufficiently accurate, precise, robust and safe for use in BSTI laboratory. Now the method is ready for routine analysis.

References:

1. The Fitness for Purpose of Analytical Methods, A Laboratory Guide to Method Validation and Related Topics, Second Edition 2014

2. International Standard ISO 22855, Fruit and vegetable products- Determination of Benzoic acid and Sorbic acid concentrations-High performance liquid chromatography method