Supplementary Information (SI)

High-Throughput Experimentation for the Application in Oxidation Reactions: Solid Dispensing Approach for Miniaturised Reactions

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1 Experimental

1.1 Materials

All the reagents used were purchased from Merck/Sigma-Aldrich and used as received without further purification. The reagents used include styrene (≤99%), benzaldehyde (≥99%), ethyl bromide (C_2H_5Br, 99%), benzyl bromide (C_6H_7Br, 98%), tert-butyl hydroperoxide (TBHP, 70% in H_2O), and copper(II) sulphate pentahydrate ($CuSO_4.5H_2O$, ≥98%). Bases used are sodium hydrogen carbonate ($NaHCO_3$, 99.5%), potassium carbonate (K_2CO_3, 99%), cesium carbonate (Cs_2CO_3, 97%), and sodium carbonate (Na_2CO_3, 99.5%). The solvents include acetone ($(CH_3)_2CO$) (99.9%), acetonitrile (MeCN, ≥99.8%), and dichloromethane (DCM, ≥99.8%). Decane ($C_{10}H_{22}$, ≥99%) and dodecane [$CH_3(CH_2)_{10}CH_3$] (≥99%) were used as internal standards. The inert carrier is silica (SiO_2, pore size 60 Å, 230-400 mesh particle size, 40-63 μm particle size). Various pyridine-based ligands with different electronic and steric properties were investigated, see Table SI 1.

Table SI 1: Pyridine ligands with their labels (numbering) used for the oxidation reactions of benzaldehyde.

Labels	Pyridine ligands
P1	2,2'-Bipyridyl, ≥99%
P2	1,10-Phenanthroline, ≥99%
P3	3-Aminopyridine, 99%
P4	2-Chloro-5-nitropyridine, 99%
P5	4-Dimethyl-aminopyridine, 99%
P6	2-Hydroxyl-3-nitropyridine, 98%
P7	2-[N,N-bis(trifluoromethyl-sulfonyl)amino]pyridine, 96%
P8	2-Phenyl-pyridine, 98%
P9	2-Chloro-6-methoxy-pyridine, 98%
P10	2,6-Diacetyl-pyridine, 99%
P11	4,4'-Bipyridyl, 98%
P12	Pyridine, ≥99%

1.2 Instrumentation

1.2.1 Shimadzu gas chromatography-flame ionisation detector (GC–FID) and gas chromatography-mass spectrometry (GC–MS) QP2010

The Shimadzu GC–MS and GC–FID 2010 plus were used for qualitative and quantitative analysis of samples. The Shimadzu GC–FID 2010 plus was equipped with a Restek RTX–5

capillary column (30 m × 0.25 µm × 0.25 mm film thickness). The injection was performed in a split mode (20:1 split ratio) at 250 ℃ using nitrogen gas (25 mL/min) as a carrier gas under 102.4 kPa. Other parameters include a total flow of 19.2 mL/min; column flow of 0.77 mL/min, linear velocity of 25.0 cm/sec, and purge flow: 3.0 mL/min. Oven conditions: samples (1 µL) were analysed at 40 ℃ for 2 minutes before increasing to 280 ℃, at a rate of 12 ℃/minute, and kept constant for 5 minutes. The detector was maintained at 280 ℃ using hydrogen gas (40 mL/min) and air (400 mL/min) to maintain the flame. Data were collected at a rate of 40 msec intervals.

The Shimadzu GC–MS 2010 plus was equipped with an SLB-5ms capillary column (30 m × 0.25 µm × 0.25 mm film thickness). The injection was performed in a split mode (20:1 split ratio) at 250 ℃ using helium as carrier gas. Column pressure: 102.4 kPa; total flow of 41.3 mL/min, flow rate of 1.82 mL/minute, linear velocity of 48.7 cm/sec, and purge flow of 3.0 mL/min. Oven conditions are similar to the GC–FID method. Mass spectrometry (MS) parameters: an ionisation temperature of 200 ℃ and an interface temperature of 250 ℃. Data were acquired in scan mode with an event time of 0.50 sec, a scan speed of 2000, a mass-to-charge range (m/*z*) of 20 to 1000, and a detector voltage of 0 kV.

1.2.2 3D printers

The Original Prusa i3 MK3 printer was purchased from *www.prusa3d.com*, and an Anycubic monocure SLA 3D printer was purchased from *www.amazon.com*. The labware was 3D printed using light-sensitive photopolymer resin and polylactic acid (PLA) thermoplastic filament purchased from *www.3dprintingstore.co.za.*

The Onshape (Onshape.com) Computer-Aided Design (CAD) online program was used to design objects, and the Standard Triangle Language (STL) files were sliced using the PrusaSlicer program before they were 3D printed.

1.2.3 The Opentrons (OT-2, pipetting robot)

The Opentrons (OT-2) is a liquid handling robot (pipetting robot) used for pipetting all the stock solutions of reagents to reaction vials in the 96-aluminium block used in the oxidation reactions of benzaldehyde. The robot was purchased from *www.opentrons.com*. Single and multichannel pipettes were used for pipetting purposes. The protocol used for pipetting was designed using the Opentrons protocol design app. The user-friendly app allows users to record

volumes and names of stock solutions needed for each well. The designed protocol can then be exported and imported into the Opetrons software, and all the required/recorded volumes can be pipetted where needed.

1.3 General method

1.3.1 Oxidation reactions of benzaldehyde to esters

Oxidation reactions were performed using benzaldehyde (nucleophile) with aryl halide (ethylbromine and benzylbromine) in a 96-aluminium block using four bases and twelve pyridine-based ligands. Bases include sodium hydrogen carbonate ($NaHCO_3$), potassium carbonate (K_2CO_3), cesium carbonate (Cs_2CO_3), and sodium carbonate (Na_2CO_3). The list of ligands is in Table SI 1. All solid reagents were dispensed into reaction vials using 0.2 g 3D printed transfer scoops, whereas the Opentrons (liquid pipetting robot) was used to dispense stock solutions. Figure SI 1 and Figure SI 2 show labware used during the experimental setup.

1.3.1.1 Reactions A1–A12: base Na_2HCO_3 and ligands P1–P12

In reactions vials A1–A12, ligands P1–P12 (10 mol%) on SiO_2 and Na_2HCO_3 (0.2 mmol) dispensed into acetonitrile MeCN (400 µL), reaction solvent. Afterwards, aliquots of 100 µL stock solutions of benzaldehyde (0.2 mmol), ethyl bromide (0.24 mmol) and dodecane (1 equivalent) were dispensed into the reaction mixtures. The catalyst, $CuSO_4.5H_2O$ (10 mol%) on SiO_2 into reactions. Lastly, aliquots of 100 µL of TBHP (2 equivalent) were dispensed into the reaction vials. Reaction vials were heated at 80 ℃ for 3 hours. Samplings (100 uL) were done at 0 hours (before the reaction) and after 24 hours. The reaction mixture was subjected to a small silica column to remove inorganic species. A syringe filter was used to remove solid particles in samples and diluted with DCM (1 mL) for analysis. The samples were analysed using GC-FID for quantitative purposes and GC-MS for qualitative purposes.

1.3.1.2 Reactions B1–B12: base K_2CO_3 and ligands P1–P12

In reactions vials A1–A12, ligands P1–P12 (10 mol%) on SiO_2 and K_2CO_3 (0.2 mmol) dispensed into acetonitrile MeCN (400 µL), reaction solvent. Afterwards, aliquots of 100 µL stock solutions of benzaldehyde (0.24 mmol), ethyl bromide (0.2 mmol) and dodecane (1 equivalent) were dispensed into the reaction mixtures. The catalyst, $CuSO_4.5H_2O$ (10 mol%) on SiO_2 into reactions. Lastly, aliquots of 100 µL of TBHP (2 equivalent) were dispensed into the reaction vials. Reaction vials were heated at 80 ℃ for 3 hours. Samplings (100 uL) were done at 0 hours (before the reaction) and after 24 hours. The reaction mixture was subjected to

a small silica column to remove inorganic species. A syringe filter was used to remove solid particles in samples and diluted with DCM (1 mL) for analysis. The samples were analysed using GC-FID for quantitative purposes and GC-MS for qualitative purposes.

1.3.1.3 Reactions C1–C12: base Cs_2CO_3 and ligands P1–P12

In reactions vials A1–A12, ligands P1–P12 (10 mol%) on SiO_2 and Cs_2CO_3 (0.2 mmol) dispensed into acetonitrile MeCN (400 µL), reaction solvent. Afterwards, aliquots of 100 µL stock solutions of benzaldehyde (0.24 mmol), ethyl bromide (0.2 mmol) and dodecane (1 equivalent) were dispensed into the reaction mixtures. The catalyst, $CuSO_4.5H_2O$ (10 mol%) on SiO_2 into reactions. Lastly, aliquots of 100 µL of TBHP (2 equivalent) were dispensed into the reaction vials. Reaction vials were heated at 80 ℃ for 3 hours. Samplings (100 uL) were done at 0 hours (before the reaction) and after 24 hours. The reaction mixture was subjected to a small silica column to remove inorganic species. A syringe filter was used to remove solid particles in samples and diluted with DCM (1 mL) for analysis. The samples were analysed using GC-FID for quantitative purposes and GC-MS for qualitative purposes.

1.3.1.4 Reactions D1–D12: base Na_2CO_3 and ligands P1–P12

In reactions vials A1–A12, ligands P1–P12 (10 mol%) on SiO_2 and Na_2HCO_3 (0.2 mmol) dispensed into acetonitrile MeCN (400 µL), reaction solvent. Afterwards, aliquots of 100 µL stock solutions of benzaldehyde (0.24 mmol), ethyl bromide (0.2 mmol) and dodecane (1 equivalent) were dispensed into the reaction mixtures. The catalyst, $CuSO_4.5H_2O$ (10 mol%) on SiO_2 into reactions. Lastly, aliquots of 100 µL of TBHP (2 equivalent) were dispensed into the reaction vials. Reaction vials were heated at 80 ℃ for 3 hours. Samplings (100 uL) were done at 0 hours (before the reaction) and after 24 hours. The reaction mixture was subjected to a small silica column to remove inorganic species. A syringe filter was used to remove solid particles in samples and diluted with DCM (1 mL) for analysis. The samples were analysed using GC-FID for quantitative purposes and GC-MS for qualitative purposes.

1.3.1.5 Reactions E1–E12: base Na_2HCO_3 and ligands P1–P12

In reactions vials A1–A12, ligands P1–P12 (10 mol%) on SiO_2 and Na_2HCO_3 (0.2 mmol) dispensed into acetonitrile MeCN (400 µL), reaction solvent. Afterwards, aliquots of 100 µL stock solutions of benzaldehyde (0.24 mmol), benzyl bromide (0.2 mmol) and dodecane (1 equivalent) were dispensed into the reaction mixtures. The catalyst, $CuSO_4.5H_2O$ (10 mol%) on SiO_2 into reactions. Lastly, aliquots of 100 µL of TBHP (2 equivalent) were dispensed into

the reaction vials. Reaction vials were heated at 80 ℃ for 3 hours. Samplings (100 uL) were done at 0 hours (before the reaction) and after 24 hours. The reaction mixture was subjected to a small silica column to remove inorganic species. A syringe filter was used to remove solid particles in samples and diluted with DCM (1 mL) for analysis. The samples were analysed using GC-FID for quantitative purposes and GC-MS for qualitative purposes.

1.3.1.6 Reactions F1–F12: base K_2CO_3 and ligands P1–P12

In reactions vials A1–A12, ligands P1–P12 (10 mol%) on SiO_2 and K_2CO_3 (0.2 mmol) dispensed into acetonitrile MeCN (400 µL), reaction solvent. Afterwards, aliquots of 100 µL stock solutions of benzaldehyde (0.24 mmol), benzyl bromide (0.2 mmol) and dodecane (1 equivalent) were dispensed into the reaction mixtures. The catalyst, $CuSO_4.5H_2O$ (10 mol%) on SiO_2 into reactions. Lastly, aliquots of 100 µL of TBHP (2 equivalent) were dispensed into the reaction vials. Reaction vials were heated at 80 ℃ for 3 hours. Samplings (100 uL) were done at 0 hours (before the reaction) and after 24 hours. The reaction mixture was subjected to a small silica column to remove inorganic species. A syringe filter was used to remove solid particles in samples and diluted with DCM (1 mL) for analysis. The samples were analysed using GC-FID for quantitative purposes and GC-MS for qualitative purposes.

1.3.1.7 Reactions G1–G12: base Cs_2CO_3 and ligands P1–P12

In reactions vials A1–A12, ligands P1–P12 (10 mol%) on SiO_2 and Cs_2CO_3 (0.2 mmol) dispensed into acetonitrile MeCN (400 µL), reaction solvent. Afterwards, aliquots of 100 µL stock solutions of benzaldehyde (0.24 mmol), benzyl bromide (0.2 mmol) and dodecane (1 equivalent) were dispensed into the reaction mixtures. The catalyst, $CuSO_4.5H_2O$ (10 mol%) on SiO_2 into reactions. Lastly, aliquots of 100 µL of TBHP (2 equivalent) were dispensed into the reaction vials. Reaction vials were heated at 80 ℃ for 3 hours. Samplings (100 uL) were done at 0 hours (before the reaction) and after 24 hours. The reaction mixture was subjected to a small silica column to remove inorganic species. A syringe filter was used to remove solid particles in samples and diluted with DCM (1 mL) for analysis. The samples were analysed using GC-FID for quantitative purposes and GC-MS for qualitative purposes.

1.3.1.8 Reactions H1–H12: base Na_2CO_3 and ligands P1–P12

In reactions vials A1–A12, ligands P1–P12 (10 mol%) on SiO_2 and Na_2CO_3 (0.2 mmol) dispensed into acetonitrile MeCN (400 µL), reaction solvent. Afterwards, aliquots of 100 µL stock solutions of benzaldehyde (0.24 mmol), benzyl bromide (0.2 mmol) and dodecane (1

equivalent) were dispensed into the reaction mixtures. The catalyst, $CuSO_4.5H_2O$ (10 mol%) on SiO_2 into reactions. Lastly, aliquots of 100 µL of TBHP (2 equivalent) were dispensed into the reaction vials. Reaction vials were heated at 80 ℃ for 3 hours. Samplings (100 uL) were done at 0 hours (before the reaction) and after 24 hours. The reaction mixture was subjected to a small silica column to remove inorganic species. A syringe filter was used to solid particles in samples and diluted with DCM (1 mL) for analysis. The samples were analysed using GC-FID for quantitative purposes and GC-MS for qualitative purposes.

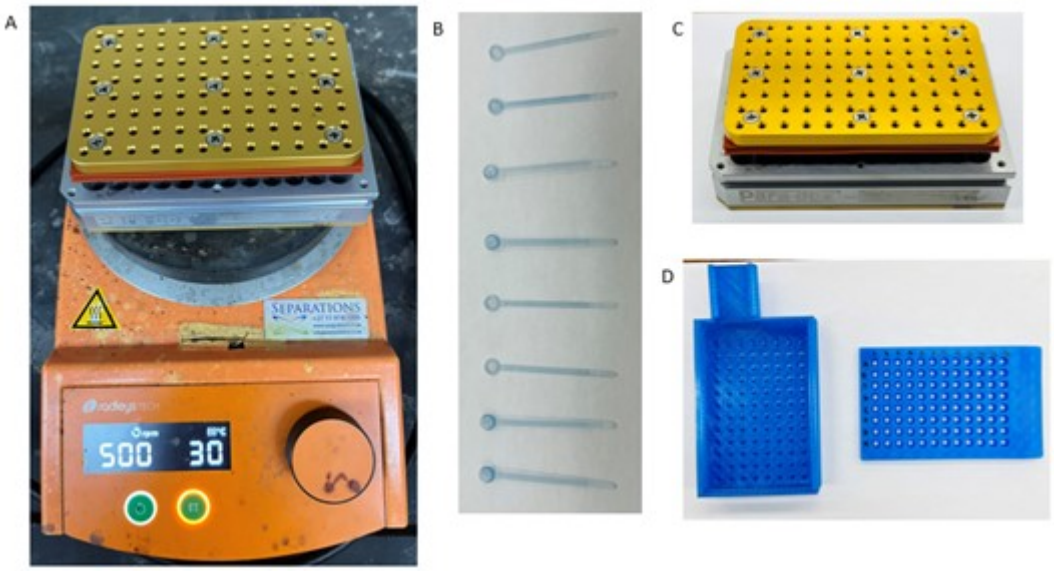


Figure SI 1: Picture of equipment used during this research: (A) 96-aluminium block on Radley magnetic stirrer, (B) 0.2 g 3D printed transfer scoops, (C) sealed 96-aluminium block with reaction vials, and (D) stirrer dispenser and funnel for dispensing reagents into 96-aluminium block.

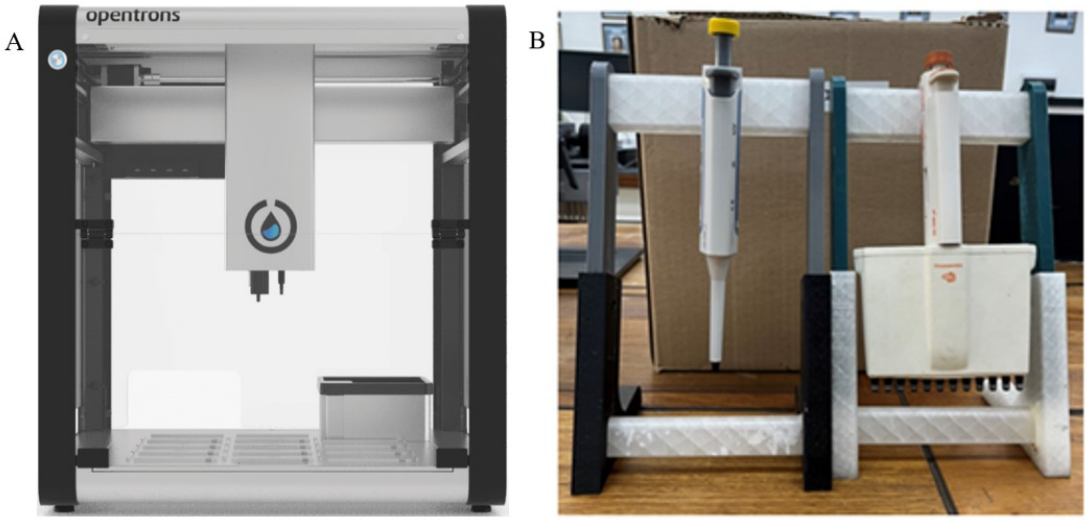


Figure SI 2: Picture of pipetting equipment: (A) the Opentrons (OT-2) from https://opentrons.com/robots/ot-2, and (B) single and multichannel micropipettes from our research lab.

1.3.2 GC–FID and GC–MS analysis

Reaction mixtures were analysed using a GC–MS (Shimadzu 2010 Plus). For the styrene oxidation, a 100 µL sample was collected from the reaction tube at 0 hours (before the start of the reaction). Afterwards, reaction mixtures were subjected to a small silica column to remove inorganic species and filtered using a syringe to remove solid particles. Then, 100 µL was taken to prepare the final samples. Samples were diluted with dichloromethane (DCM). Finally, the amount of substrate converted to products was confirmed, and the retention time of the products formed.

In the oxidation reaction of benzaldehyde, a 60 µL sample was taken at 0 hours and added to gc insets (can be used to analyse a small sample volume) for analysis without dilution for all samples. A small volume (60 µL) was taken to avoid taking too much reaction mixture before the start of the reaction, given that only 800 µL was used for reactions. After the reaction, a 100 µL sample was taken after being subjected to a small silica column and syringe filtered. The samples were also diluted for GC-MS analysis. The conversion of substrate to products was confirmed, and the formation of products.

The tables below (Table SI 2, Table SI 3, Table SI 4, Table SI 5, and Table SI 6) indicate reagents under investigation.

Table SI 2: Substrates with their respective labels used for the oxidation reactions of benzaldehyde.

Labels	Reagents	Volume of stock solutions (µL)	Volume per well (µL)
1	Benzaldehyde	304.94	100
2a	Ethylbromide	186.59	100
2b	Benzyl bromide	296.94	100

Table SI 3: Reagents dispensed as solids and stock solutions used for the oxidation reactions of benzaldehyde.

These reagents were dispensed as solids using 0.2 g transfer scoops.	These reagents were dispensed as stock solutions (100 µl) using Opentrons 2 (OT-2) or 100-1000 µl micropipette.
12 pyridine ligands	Benzaldehyde: 304.94 µL in 10 mL DMF
$CuSO_4.5H_2O$	Ethyl bromide: 186.59 µL in 10 mL DMF
$NaHCO_3$	Benzyl bromide: 296.94 µL in 10 mL DMF
K_2CO_3	TBHP: 481.94 µL in 10 mL DMF
Cs_2CO_3	Dedocane: 681.81 µL in 10 mL DMF
Na_2CO_3	

Table SI 4: List of bases used to screen the oxidation reactions of benzaldehyde.

Labels	Bases	Mass per well (g)
C1	Na_2HCO_3	0.0168
C2	K_2CO_3	0.0276
C3	Cs_2CO_3	0.0652
C4	Na_2CO_3	0.0202

Table SI 5: List of pyridine ligands coated on SiO_2 used to screen the oxidation reactions of benzaldehyde.

Labels	Ligands	Amount of ligands	Mass of SiO_2 (g)
P1	2,2'-Bipyridyl	0.0180 g	4.9820
P2	1,10-Phenanthroline	0.0155 g	4.9845
P3	3-Aminopyridine	0.0094 g	4.9906
P4	2-Chloro-5-nitropyridine	0.0159 g	4.9841
P5	4-Dimethyl-aminopyridine	0.0122 g	4.9878
P6	2-Hydroxyl-3-nitropyridine	0.0109 g	4.9891

P7	2-[*N,N*-bis(trifluoromethyl-sulfonyl)amino]pyridine	0.0358 g	4.9642
P8	2-Phenyl-pyridine	14.50 µL	4.9845
P9	2-Chloro-6-methoxy-pyridine	12.00 µL	4.9856
P10	2,6-Diacetyl-pyridine	0.0163 g	4.9837
P11	4,4'-Bipyridyl	0.0156 g	4.9844
P12	Pyridine	8.10 µL	4.9921

Table SI 6: Catalyst used to screen the oxidation reactions of benzaldehyde.

Catalyst	Mass of catalyst (g)	Mass of SiO_2 (g)
$CuSO_4.5H_2O$ on SiO_2	0.0050	9.9950

3 Results

3.1 Appendices: GC-MS spectra

3.1.1 Formulae used for the calculations of substrate conversions and product selectivity.

Conversions of substrate were calculated using 0-hour and 24-hour sample GC-FID results. In each sample, peak intensity/area was used for the calculations together with peak intensity/area of internal standard (dodecane). The formula is defined as:

$$C_s = \left[1 - \frac{\left(\frac{Isubstrate}{Idodecane}\right)24\ hrs}{\left(\frac{Isubstrate}{Idodecane}\right)0\ hr}\right] \times 100$$ ----------equation 1

where C = the conversion rate of the substrate

$I_{substrate}$ = the intensity of substrate (at 24 hours and 0 hours)

$I_{dodecane}$ = the intensity of decane (24 hours and 0 hours)

Product selectivity was determined based on the following formulae:

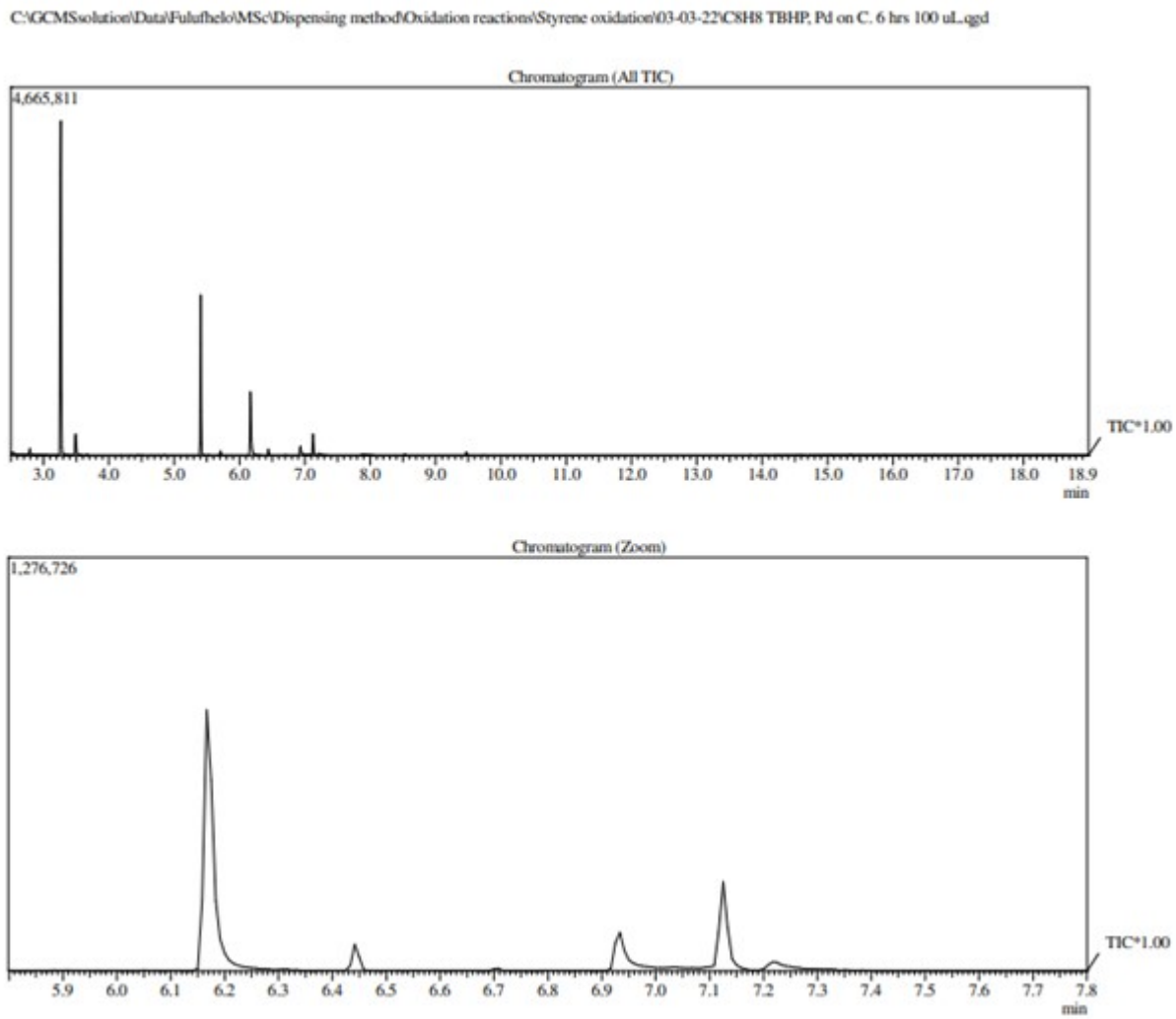
$$P_s = \frac{Ip1}{\Sigma(Ip1 + Ip2)} \times 100$$----------equation 2

Where I_{p1} = the intensity of product P_1

I_{p2} = the intensity of product P_2

3.1.2 Oxidation reaction of styrene

The oxidation reaction of styrene formed four products: (R)-styrene oxide, phenylacetaldehyde, benzaldehyde, and benzoic acid. The GC-MS results confirming the products' structures are shown in Figure SI 3-Figure SI 11. The chromatogram shows peaks at 6.167 min, 6.933 min, 7.125 min, and 7.892 min (retention time) for benzaldehyde, phenylacetaldehyde, (R)-styrene oxide, and benzoic acid, respectively. The mass spectrum shows the products' molecular masses of 106-, 120-, 119-, and 122- g/mol for the products



obtained.

Figure SI 3: Chromatogram for the 24-hour sample. The peaks at retention times of 6.167-, 6.933-, 7.125-, and 7.892- min (retention time) for benzaldehyde, phenylacetaldehyde, (R)-styrene oxide, and benzoic acid, respectively, from the oxidation reaction of styrene.

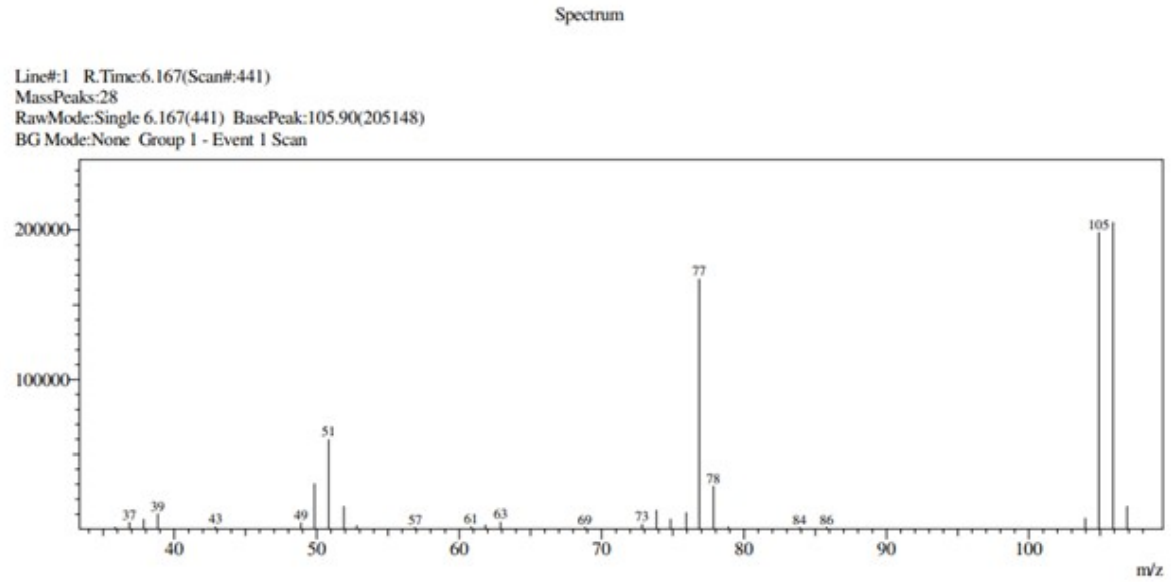


Figure SI 4: Mass spectrum indicating 106 g/mol molecular mass for benzaldehyde product.

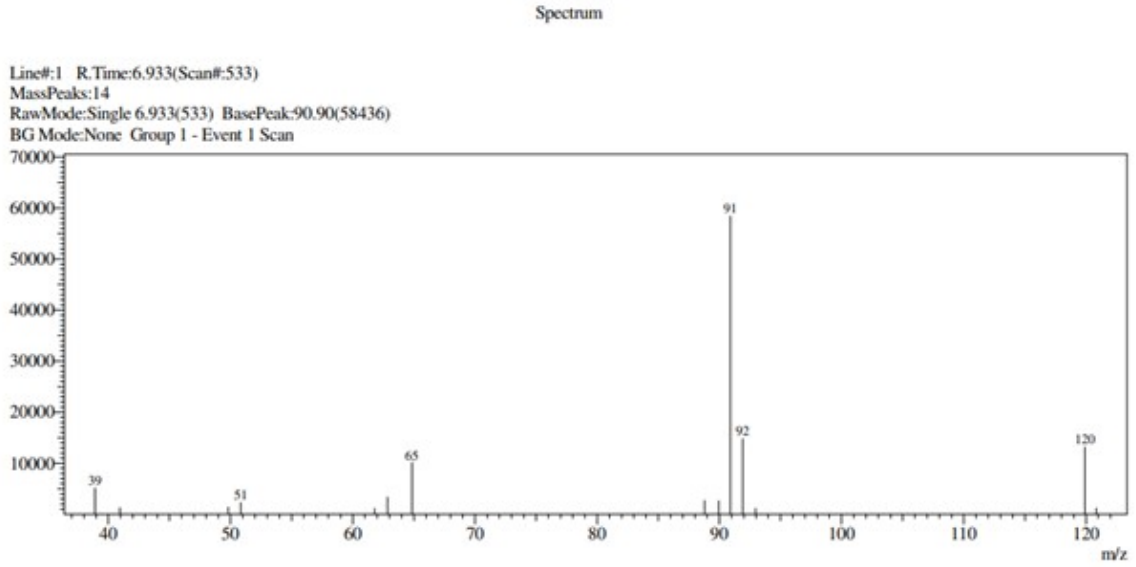


Figure SI 5: Mass spectrum indicating 120 g/mol molecular mass for phenylacetaldehyde product.

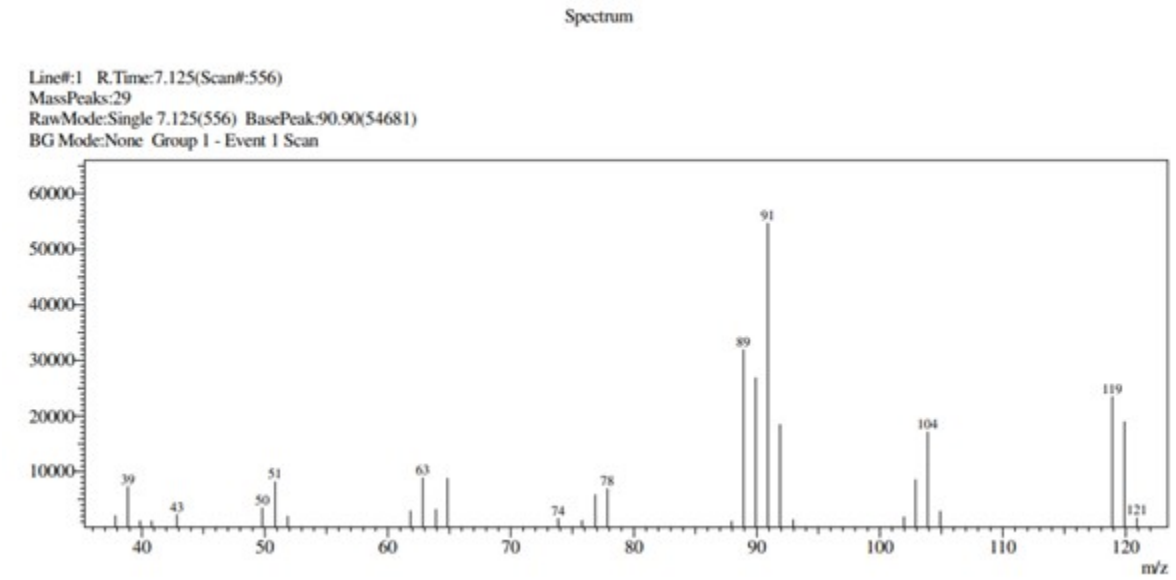


Figure SI 6: Mass spectrum indicating 119 g/mol molecular mass for (R)-styrene oxide product.

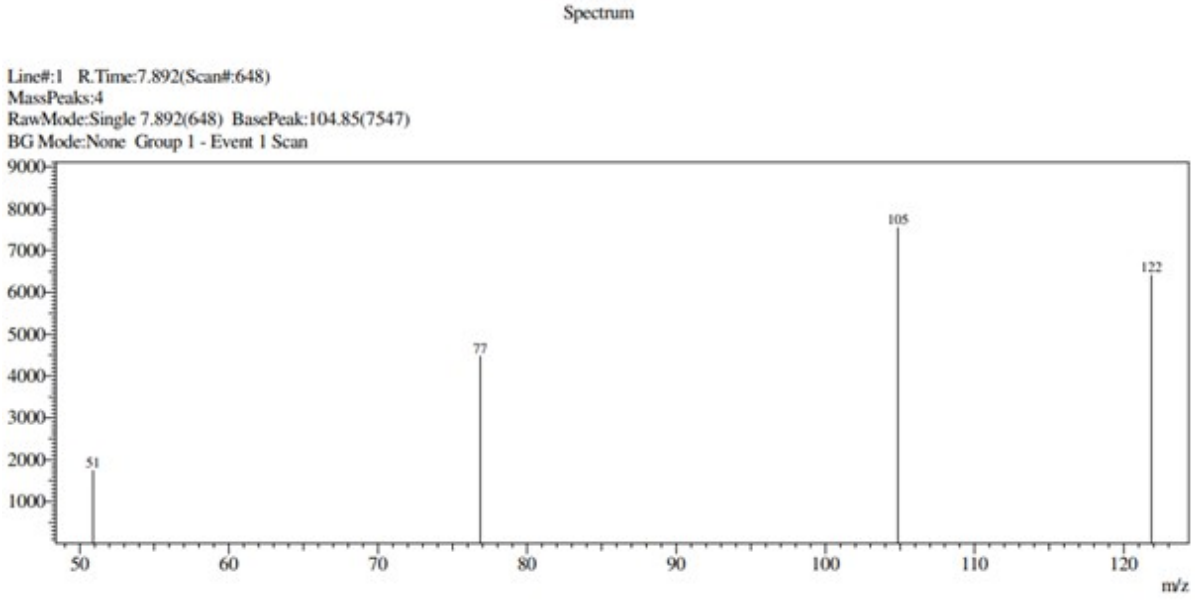


Figure SI 7: Mass spectrum indicating 122 g/mol molecular mass for benzoic acid product.

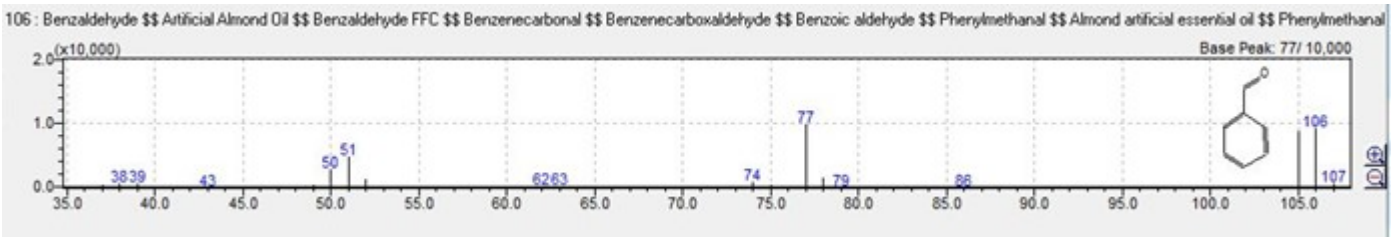


Figure SI 8: Screenshot indicating the structure of the benzaldehyde product.

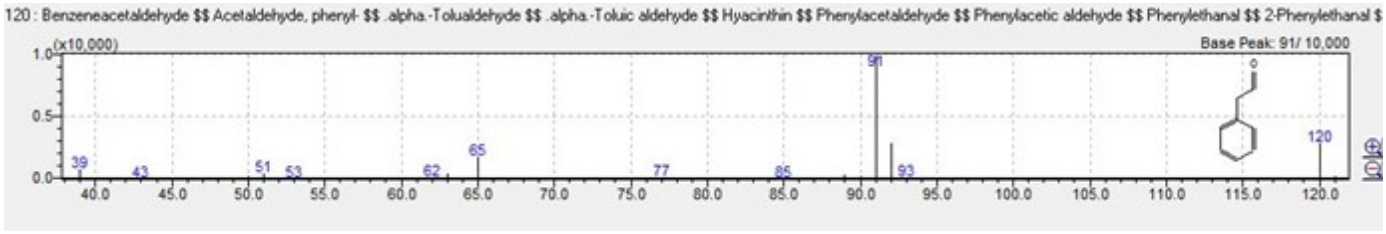


Figure SI 9: Screenshot indicating the structure of the phenylacetaldehyde product.

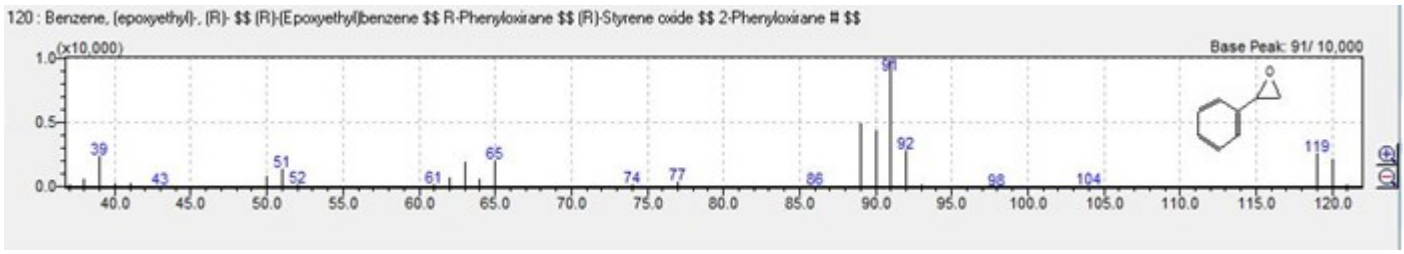


Figure SI 10: Screenshot indicating the structure of the (R)-styrene oxide product.

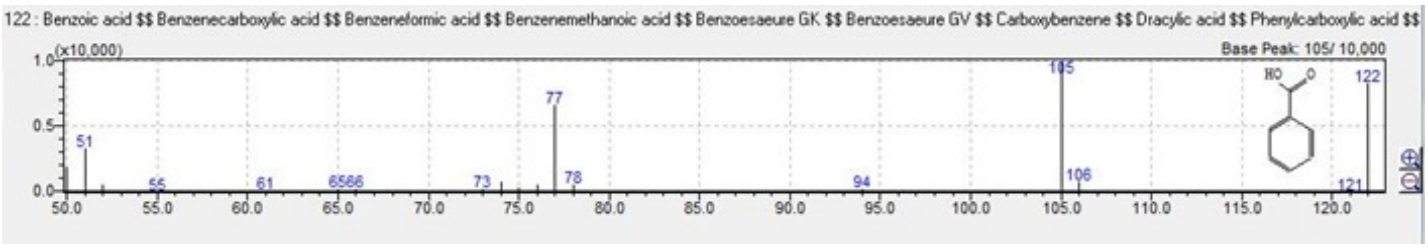


Figure SI 11: Screenshot indicating the structure of the benzoic acid product.

3.1.3 Oxidative esterification of benzaldehyde

Copper-based oxidation reactions of benzaldehyde with ethyl benzene and benzyl bromide form ethyl benzoate and benzyl benzoate.

3.1.3.1 Oxidative esterification of benzaldehyde (1) with ethyl bromide (2a) to ethyl benzoate and benzoic acid

The GC-MS results confirming ethyl benzoate and benzoic acid structures are shown in Figure SI 12, Figure SI 13, Figure SI 14, and Figure SI 15. The chromatogram shows peaks at 7.892 min and 10.250 min (retention time) for ethyl benzoate and benzoic acid, respectively. The mass spectrum shows the products' molecular masses of 122 g/mol and 150 g/mol.

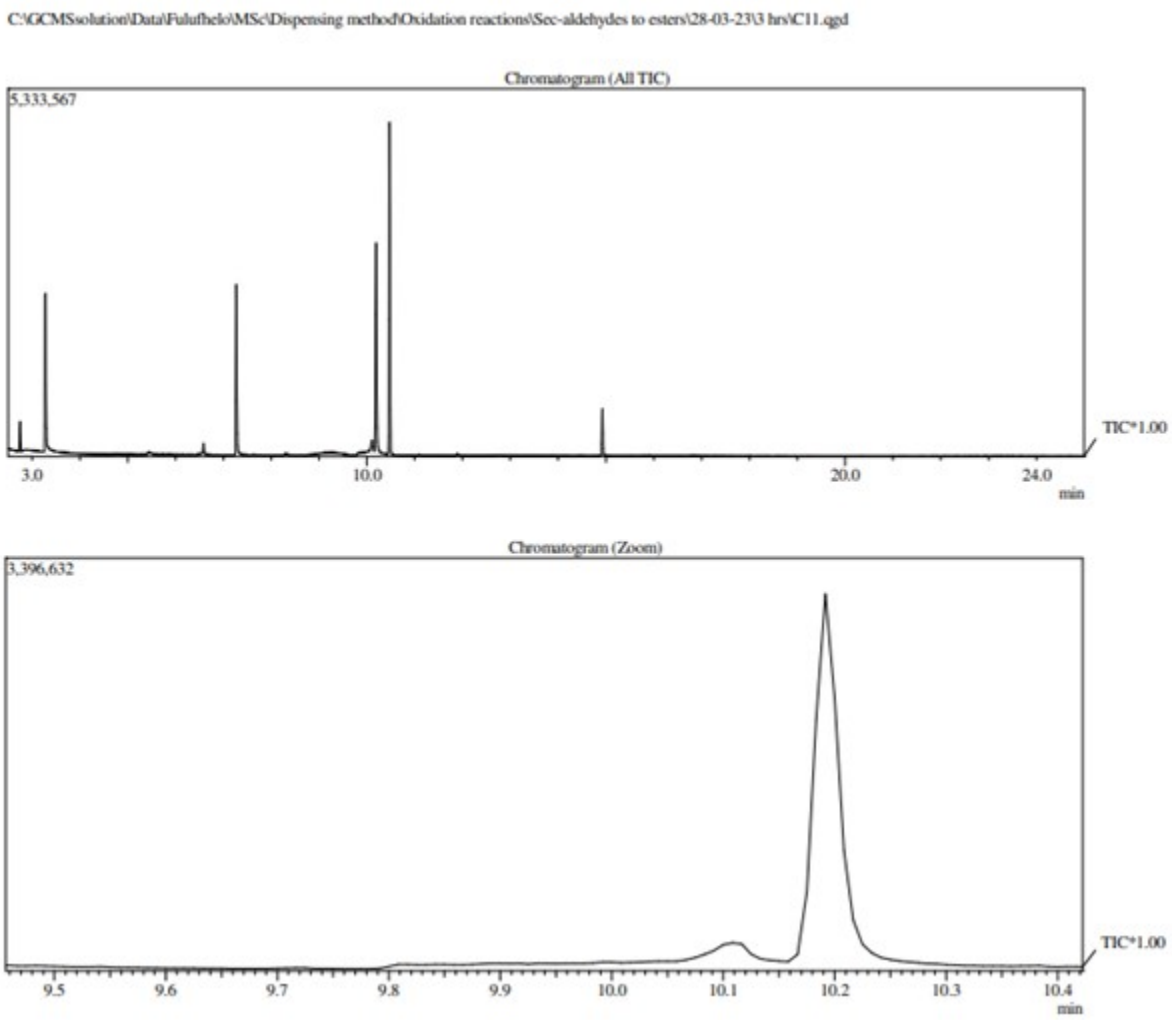


Figure SI 12: Chromatogram for the 24-hour sample. The peak at retention times of 7.892 and 10.250 min is for benzoic acid and benzyl benzoate products from the oxidation reactions of benzaldehyde with ethylbromide.

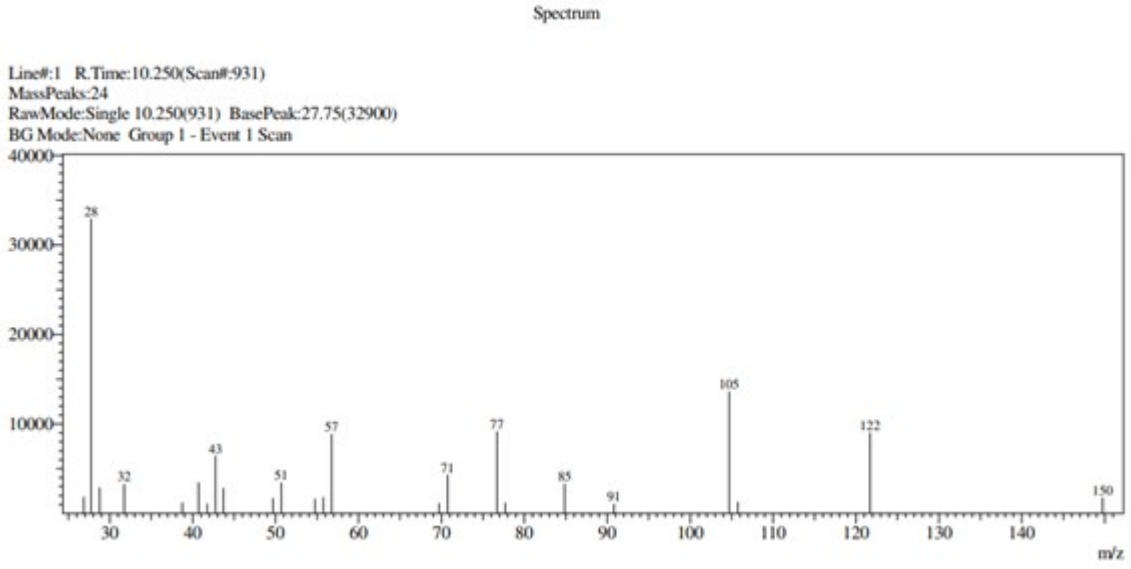


Figure SI 13: Mass spectrum indicating 122 g/mol and 150 g/mol molecular mass for benzoic acid and ethyl benzoate products.

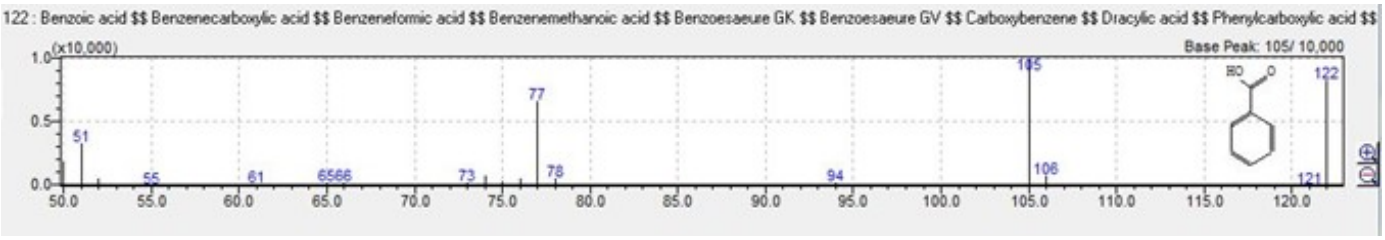


Figure SI 14: Screenshot indicating the structure of the benzoic acid product.

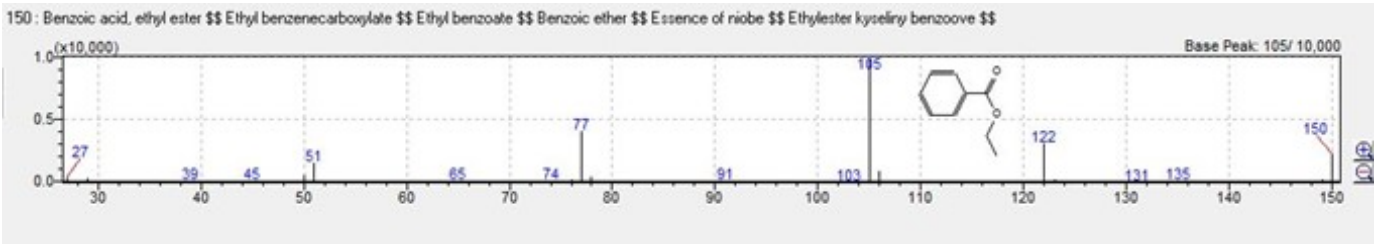


Figure SI 15: Screenshot indicating the structure of the ethyl benzoate product.

3.1.3.2 Oxidative esterification of benzaldehyde (1) with benzyl bromide (2b) to benzyl benzoate

The GC-MS results confirming benzyl benzoate structure are shown in Figure SI 16, Figure SI 17and Figure SI 18. The chromatogram shows the peak at 16.708 min (retention time) for benzyl benzoate. The mass spectrum shows the products' molecular masses of 212 g/mol.

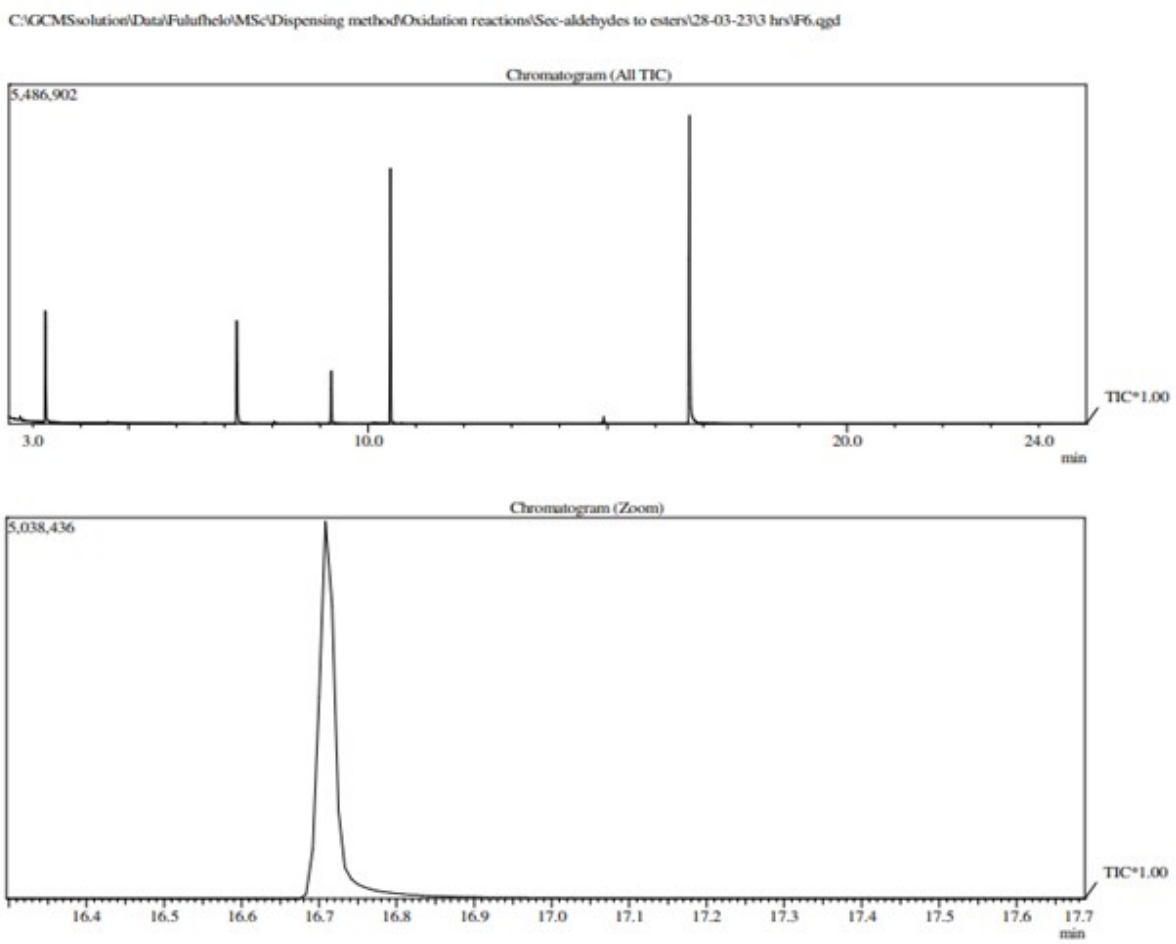


Figure SI 16: Chromatogram for the 24-hour sample. The peak at the retention time of 16.708 min is for the benzyl benzoate product from oxidation reactions of benzaldehyde with benzyl bromide.

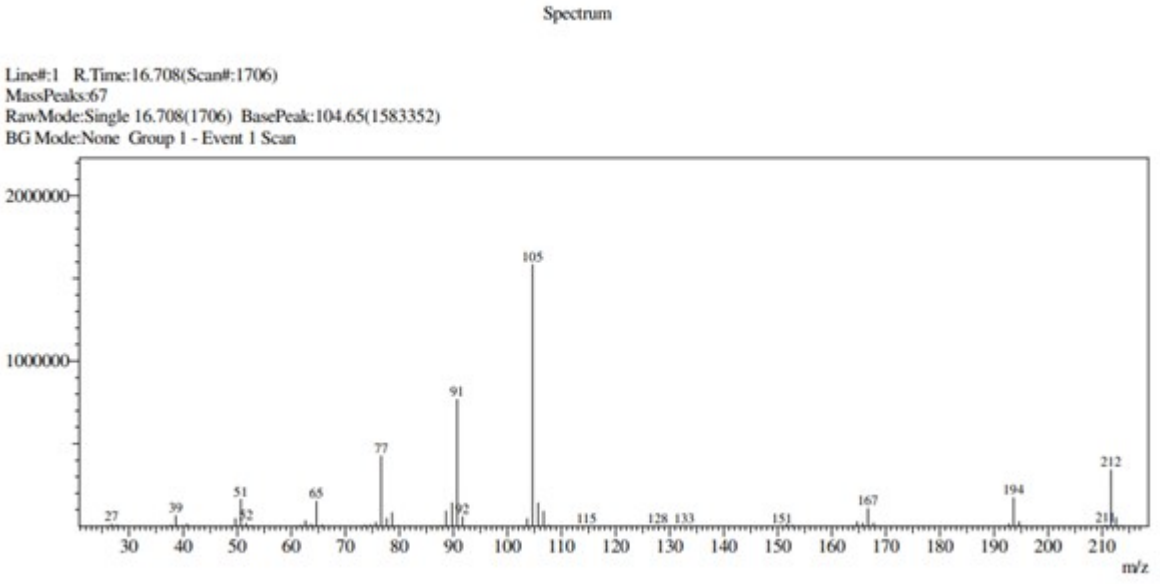


Figure SI 17: Mass spectrum indicating the 212 g/mol molecular mass of benzyl benzoate product.

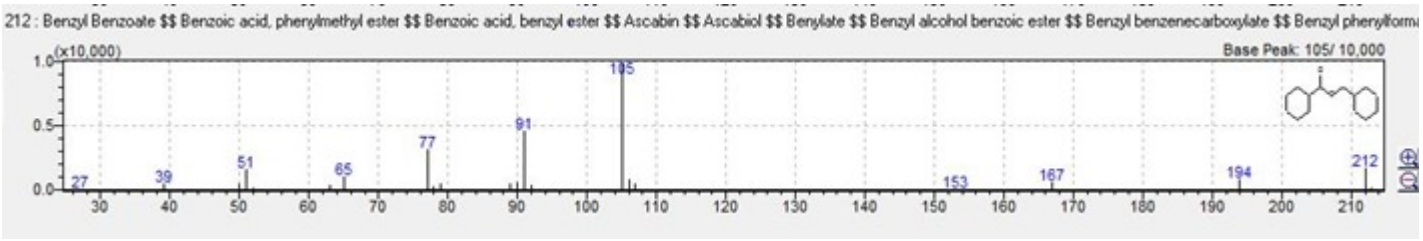


Figure SI 18: Screenshot indicating the structure of the benzyl benzoate product.