Separation of Phenols using Thin Layer Chromatography

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Abstract

The purpose of this research was to determine the constituents of three unknowns, using thin layer chromatography. Running the 5 standards and three unknown mixtures, they were identified. Unknown 4a had two compounds. The first with R_f values of 0.26 in MP1, 0.45 in MP2, and 0.82 in MP3 – which closely matched the R_f values for phenol. The second compound in 4a had 0.92, 0.86, 0.97 R_f values closely matching 2-nitrophenol. Unknown mixture 4b had two compounds, the first with R_f values of 0.01 in MP1, 0.06 in MP2, and 0.30 in MP3 – which closely matched hydroquinone's R_f values. The second compound in 4b had 0.25, 0.44, 0.82 R_f values closely matching phenol. Unknown mixture 4c had two compounds, the first with R_f values of 0.01 in MP1, 0.07 in MP2, and 0.30 in MP3 – which closely matched hydroquinone's R_f values. The second compound in 4c had 0.06, 0.18, 0.46 R_f values closely matching 4-nitrophenol.

The identification of the 4a unknowns were phenol and 2-nitroophenol. The identification of the 4b compounds were hydroquinone and phenol, and for 4c the compounds were hydroquinone and 4-nitrophenol. The 4a, 4b, and 4c sample retention factors, from all three mobile phases, aligned well with those of the standards.

1.0 Introduction

Thin layer chromatography (TLC) is a category of liquid chromatography where the stationary phase (SP) is a thin layer usually adhering to a flat material (plate), where the analyte is eluted via a mobile phase (MP) solvent, traveling through the SP via capillary action. To create a TLC plate, SP material is applied to a plate, such as glass, aluminum, or solvent resistant plastic (though paper can be used as the SP without a supporting plate). As in other liquid chromatography, the analyte will approach equilibrium, between the stationary phase and the mobile phase, as the mobile phase through the SP. It is run in a sealed environment to prevent evaporation of the mobile phase during elution.

Like other forms of chromatography, the differential competition between attraction to the stationary phase and flowing in/with the solvent of the mobile phase (MP) allows separation of different components, often based on their polarity and the polarity of the SP and MP[1]

The stationary phase particles, or paper, are/is extremely porous. The porosity provides a high surface area, allowing for a large reaction space. The high surface area is responsible for most of the analyte retention. The SP substrates are typically silica gel, alumina, paper or charged polymeric phases. In addition, silica gel or alumina can be treated to alter the solute affinity. By choosing an appropriate stationary and mobile phase, fine tuning of the analyte separation can usually be attained.

While thin layer chromatography can lack the precision and selectivity of HPLC or GC, it does provide a good tool for initial assessment of MP/SP use in separation of analytes requiring little in the way equipment and preparation. It has advantages over the other chromatographic techniques in its low instrumentation cost, the ability of making simultaneous separations, and a shorter analysis time. [2]

2.0 Experimental

2.1 Reagents:

The following chemicals were used in the experiments described in this paper:

For TLC elution: methylene chloride: ≥99.5%, Sigma Aldrich, St. Louis, MO, *CAS# 75-09-2;* petroleum ether: ACS certified, Fisher Chemical, Fair Lawn, NJ, *CAS# 8032-32-4*; diethyl ether: lab grade, Fisher Chemical, Fair Lawn, NJ, *CAS# 60-29-7*;

As standards and use in unknowns: hydroquinone: 99+%, Aldrich Chemical Co., Milwaukee, WI, *CAS# 123-31-9;* 2-bromophenol: 98%, Aldrich Chemical Co., Milwaukee, WI, *CAS# 95-56-7;* 2-nitrophenol: 99%, Acros Organics, New Jersey, *CAS# 88-75-7;* 4-nitrophenol: 98%, Acros Organics, New Jersey, *CAS# 100-02-7;* and Phenol: 99%, Fisher Scientific, Fair Lawn, NJ, *CAS# 108-95-2*.

The standard solutions were 1% w/v in methanol. The unknowns were of unknown concentration.

2.2 Equipment:

The materials used include: TLC Glass Plates- Silica Gel 60 F254 (5x10cm), Merck KGaA, Darmstadt, Germany; Capillary Tubes- 5 microliter calibrated pipets, VWR International; UV lamp- Spectroline ENF-280C handheld UV lamp, Spectronics Corp., Westbury, NY.; 3 400 ml Beakers- Thermo Fisher Scientific, Waltham, MA; and 3 Watch glasses- Thermo Fisher Scientific, Waltham, MA.

2.3 Experimental Conditions:

This was performed at room temperature, at approximately 16 °C, in covered beakers.

2.4 Procedures:

Three silica-gel plates were obtained, then a horizontal line was drawn, in pencil, at approximately a centimeter from the bottom and at ½ a centimeter from the top of the plate. Along the bottom line, eight evenly spaced pencil marks were made to designate where the samples would be deposited.

A capillary tube was used to draw up a small amount of sample. That was used to apply to one of the marks on each plate. The same cardinal mark on each was used for a specific sample. The positions used were the first mark for the phenol control, 2nd for the hydroquinone control, 3rd for the 2-bromophenol control, 4th for the 2-nitrophenol control, 5th for the 4-nitrophenol control, 6th for the unknown 4a, 7th for the unknown 4b, 8th for the unknown 4c.

After each plate had a small dot of sample applied to the correct position, the process was repeated to get more sample onto its designated spot.

In each of three beakers, a solvent mixture was placed, as the mobile phase, to a depth of about 4 mm. MP1 (methylene chloride/petroleum ether 80/20) was in the first, MP2 (petroleum ether/diethyl ether (80/20) was in the second, and MP3 (petroleum ether/diethyl ether (60/40) was in the third. Each beaker was covered with a watch glass once solvent was added. Time was given to allow the solvents to saturate the air in the beakers.

Prior to running elution, the spots were checked under the UV light to ensure they showed up.

3.0 Results and Discussion

3.1 Procedure:

Each of the silica gel slides were placed in a beaker containing one of the solvent mixtures and the watch glasses placed back over the top.

The elution was allowed to proceed. When the solvent made it to the top line, each of the TLC slide was removed and the top marked with the solvent mix it was in. On drying, the slides were taken to the UV light and the samples were marked. The distances were read and recorded.

Each standard used contain one of these, each: hydroquinone: 99+%, Aldrich Chemical Co., Milwaukee, WI, *CAS# 123-31-9;* 2-bromophenol: 98%, Aldrich Chemical Co., Milwaukee, WI, *CAS# 95-56-7;* 2-nitrophenol: 99%, Acros Organics, New Jersey, *CAS# 88-75-7;* 4-nitrophenol: 98%, Acros Organics, New Jersey, *CAS# 100-02-7;* and Phenol: 99%, Fisher Scientific, Fair Lawn, NJ, *CAS# 108-95-2,* as a 1% w/v solution.

The calculation of the retention factor R_f is:

Equation 1 Retention factor calculation

$\mathbf{R_f = d_1 / d_0}$ = distance traveled by sample / distance traveled by solvent[3]

Example 0.29 = 22.0 / 76.5

3.2 Data:

Silica gel consists of silica exposing silanol groups in the possible configurations below. The OH groups of the silanol groups allow for hydrogen bonding and form a polar, slightly acidic structure.

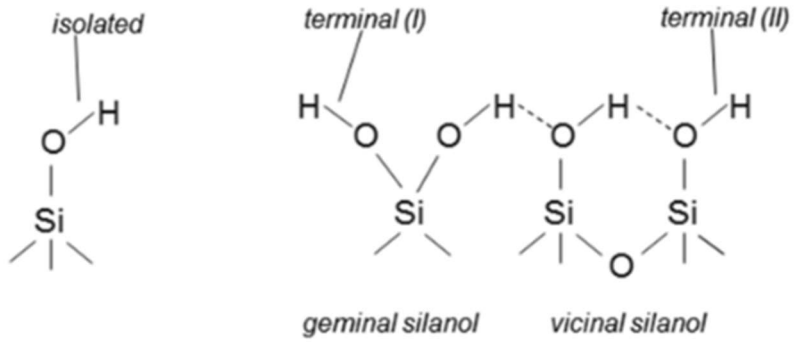

Figure 1 Silanol structure, in various configurations[4]

The structure of all phenols is that of a benzene ring with at least one hydroxyl group attached. This is an acidic, polar molecule that can and will hydrogen bond to silanol hydroxyls.

Figure 2 Molecular structures of phenol, hydroquinone, 2-bromophenol, 2-nitrophenol, and 4-nitrophenol

The constituents of the standards and unknowns are phenols of one form or another. Aside from phenol, there is hydroquinone, a phenol with a para hydroxyl group, 2-bromophenol is the base phenol molecule with a bromine in the number two position (ortho), 2-nitrophenol is analogous to 2-bromophenol, but with a nitro group instead of a bromine, and 4-nitrophenol is a phenol with a nitro in the number 4 (para) position.

Each of these molecules can hydrogen bond to silanol hydroxyls and the various electron withdrawing effects of each group affect the hydroxyls polarity and acidity.

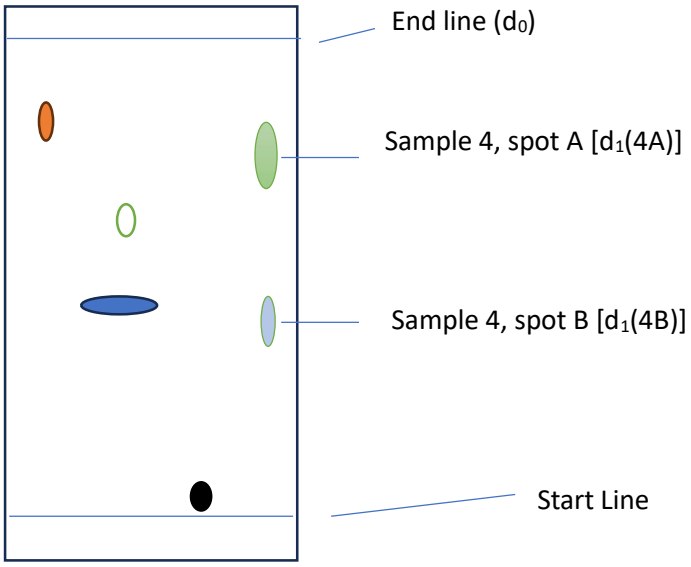

Figure 3 Example TLC plate with colored ovals representing eluted samples.

An example is shown in Figure 3. They have been colored to help with the visualizations. In the above plate, the start line is marked. The end line, the point where the MP reached when the elution was stopped, is at about 76 mm past the start line. This is considered d_0. If we focus on the far right, sample spots marked 4A and 4B, we see that they have eluted to 34 and 20 mm from the bottom, or more importantly, 30 and 16 mms from the start line.

The blue spot is meant to show two analytes eluting at the same distance. If done right next to each other, they can appear as a single spot. If a spot is very long (in the direction of travel), it indicates significant tailing. This can have multiple causes, but overloading of the analyte is a common cause.

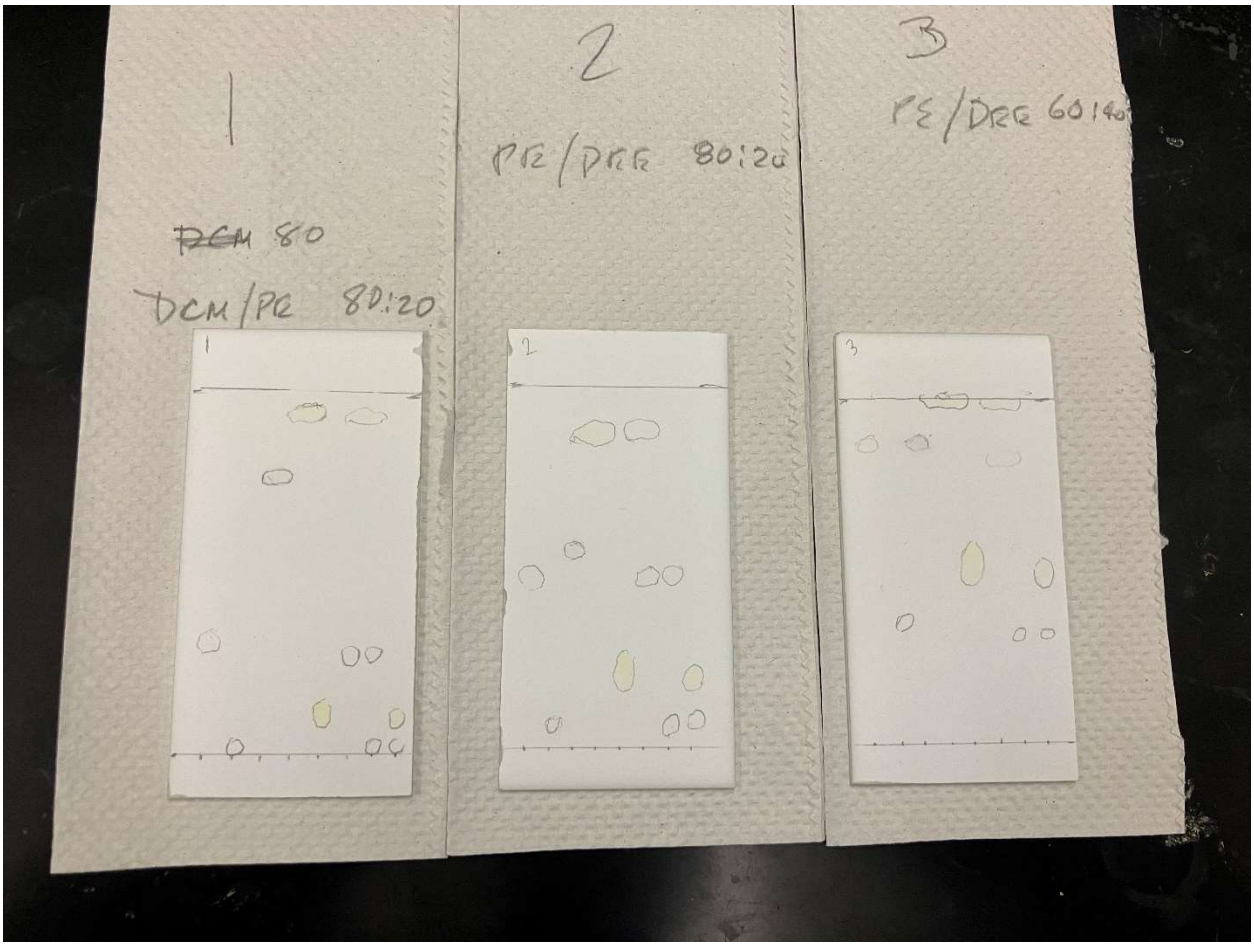

Figure 4 Three TLC plates, developed and visualized.

For Sample 4A, using the formular $R_f = d_1 / d_0$, to compute the R_f value. Thus $R_f = 20.0/76.5 = 0.26$. For Sample 4B, performing the same operation with 4Bs distance traveled, the $R_f = 1/78 = 0.01$. If the same MP and SP are used for another TLC elution, the same compounds should get retention factors very close to these retention factors.

Figure 4 are the eluted samples. Each eluted sample has been circled to allow for measurements. Visualization was done via UV light. Ultraviolet light is absorbed by UV, preventing the fluorescent component in the substrate from glowing, showing as a dark spot. This can be seen in Figure 1, below. The visualization allows marking of the samples for the taking of measurements, later.

The retention factor is the ratio of the distance the analyte travels to that of the mobile phase. It gives a good window into the equilibrium seen between the analyte and the SP and analyte and the MP. The stronger the analyte's relative attraction to the SP vs the MP, the smaller the R_f.

Table *1* through Table 6 delineate the measurements and computed retention factors, for standards and unknowns in this research.

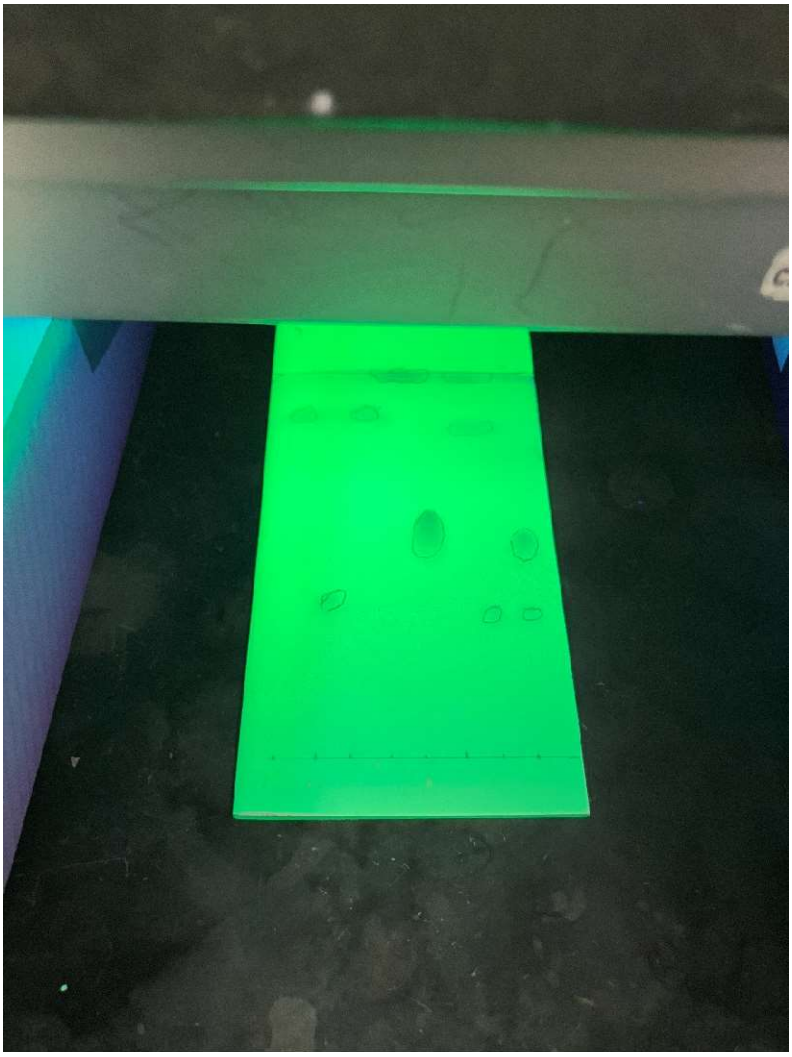

Figure 5 Visualization under ultraviolet light

Table 1 MP1 Standard distances and computed retention factor

Methylene chloride /petroleum ether (80/20)	**phenol**	**hydroquinone**	**2-bromo phenol**	**2-nitro phenol**	**4-nitro phenol**
retention distance, d_1 (cm)	2.2	0.1	5.6	7.2	0.8
d_0	7.65	7.65	7.65	7.65	7.65
Retention factor (R_f)	0.30_2	0.01	0.73	0.93	0.10

Table 2 MP2 Standard distances and computed retention factor

Petroleum ether /Diethyl ether (80/20)	**Phenol**	**Hydroquinone**	**2-bromo phenol**	**2-nitro phenol**	**4-nitro phenol**
retention distance, d_1 (cm)	3.5	0.4	4.0	6.7	1.5
d_0	7.8	7.8	7.8	7.8	7.8
Retention factor (R_f)	0.45	0.05	0.51	0.86	0.19

Table 3 3 MP3 Standard distances and computed retention factor

Petroleum ether /Diethyl ether (60/40)	**Phenol**	**hydroquinone**	**2-bromo phenol**	**2-nitro phenol**	**4-nitro phenol**
retention distance, d_1 (cm)	6.4	2.5	6.4	7.5	3.7
d_0	7.6	7.6	7.6	7.6	7.6
Retention factor (R_f)	0.84	0.33	0.84	0.99	0.49

Table 4 Mobile Phase #1 Unknowns

Methylene chloride /petroleum ether (80/20)	**4a spot 1**	**4a spot 2**	**4b spot 1**	**4b spot 2**	**4c spot 1**	**4c spot 2**
retention distance, d_1 (cm)	2.0	5.3	0.9	7.1	0.2	7.2
d_0	7.65	7.65	7.65	7.65	7.65	7.65
Retention factor (R_f)	0.26_9	0.66_3	0.11_3	0.89_1	0.02_5	0.90_6

Table 5 Mobile Phase #2 Unknowns

Petroleum ether /Diethyl ether (80/20)	**4a spot 1**	**4a spot 2**	**4b spot 1**	**4b spot 2**	**4c spot 1**	**4c spot 2**
retention distance, d_1 (cm)	3.7	4.5	1.5	7.3	0.4	7.3
d_0	7.8	7.8	7.8	7.8	7.8	7.8
Retention factor (R_f)	0.46_3	0.56_3	0.18_9	0.91_8	0.05_1	0.92_4

Table 6 Mobile Phase #3 Unknowns

Petroleum ether /Diethyl ether (60/40)	**4a spot 1**	**4a spot 2**	**4b spot 1**	**4b spot 2**	**4c spot 1**	**4c spot 2**
retention distance, d_1 (cm)	5.8	5.8	3.2	7.5	1.7	7.5
d_0	7.6	7.6	7.6	7.6	7.6	7.6
Retention factor (R_f)	0.72_9	0.72_9	0.40_3	0.94_3	0.21_4	0.94_3

Examination of Table 7 and Table 8 how the retention factors for the standards match those of the unknowns, allowing for identification of the unknowns of unknown #4.

Table 7 Table of retention factors

ID \ R_f Values	Mobile Phase #1	Mobile Phase #2	Mobile Phase #3
Phenol	0.29	0.45	0.84
Hydroquinone	0.01	0.05	0.33
2-bromophenol	0.73	0.51	0.84
2-nitrophenol	0.93	0.86	0.99
4-nitrophenol	0.10	0.19	0.49
4a – 1	0.26	0.45	0.82
4a – 2	0.92	0.86	0.97
4b – 1	0.01	0.06	0.30
4b – 2	0.25	0.44	0.82
4c – 1	0.01	0.07	0.30
4c – 2	0.09	0.18	0.46

Examination of Table 7 and Table 8 show unknown 4a contains phenol (1) and 2-nitrophenol (2); unknown 4b contains hydroquinone (1) and 2-phenol (2); and unknown 4c contains hydroquinone (1) and 4-nitrophenol (2).

Table 8 Rf Matching table

ID \ R_f Values	Mobile Phase #1	Mobile Phase #2	Mobile Phase #3
Phenol	0.29	0.45	0.84
4a-1	0.26	0.45	0.82
Hydroquinone	0.01	0.05	0.33
4b-1	0.01	0.06	0.30
2-nitrophenol	0.93	0.86	0.99
4a-2	0.92	0.86	0.97
phenol	0.29	0.45	0.84
4b-2	0.26	0.45	0.82
Hydroquinone	0.01	0.05	0.33
4c-1	0.01	0.06	0.30
4-nitrophenol	0.10	0.19	0.49
4c-2	0.09	0.18	0.46

Table 8 , above, shows the matching between knowns and unknowns, by similar R_f profiles in the three mobile phase mixtures. This forms the identification justification for the unknowns.

In examining the R_f values of the standards, a pattern appears not unlike ones seen in gas chromatography. While boiling point isn't a factor, polarity becomes very important, more specifically the polarity of the MP and the SP, relative to the analyte.

Reflected in the R_f values, the polarities of the analytes follow this progression:

2-nitrophenol < 2-bromophenol < phenol < 4-nitrophenol < hydroquinone.

The less polar the analyte, the less it is attracted to the stationary phase and the farther the solvent elutes it. The more polar the solvent mixture there is an increase in elution distance with polar analytes, as would be expected given the more polar solvent can compete better with the polar silica gel. The two nitrophenols were structural isomers and would initially be assumed to have similar polarities. 2-nitrophenol has a lower polarity, due to the intramolecular hydrogen bonding of the adjacent nitro and hydroxyl group. It's geometrical isomer, 4-nitrophenol has a few times as much distance between those functional groups, preventing any intramolecular hydrogen bonding, so has a greater polarity. Hydroquinone has two hydroxyl groups para to each other preventing intramolecular hydrogen bonding, so it is the most polar and the most retained of all the analytes.

When the polarities of the analytes and the solvents are considered, the order of the retention factors seen is in line with the chromatographic theory.

3.3 Error Analysis

Error analysis of results would not produce meaningful results, due to the limited repetitions.

4.0 Conclusion

The thin layer chromatographs were run without a problem. All eluted compounds were clearly visible under the ultraviolet lamp and there was not much tailing in the samples.

Given the unique fingerprints, or R_f value patterns for the unknowns when eluted in the different mobile phase mixtures, the identification was straightforward. Adding to the confidence in the identification was the limited number of possible unknowns and that no compounds had a similar retention factor pattern, when run with the three mobile phase mixtures. Unknown 4a was identified as phenol and 2-nitroophenol; 4b as hydroquinone and phenol, and 4c as 4-nitrophenol and hydroquinone.

A strong correlation between known and identified unknown R_f fingerprints formed the basis of identification, with most R_f values deviating no more than 0.01 from the knowns.

5.0 References

[1] Vitha, M, *Chromatography, Principles and Instrumentation*, John Wiley & Sons, inc., 2017, pp 147-149

[2] Mohammad, A., Khan, M., Ullah, Q., and Mohammad, F., Effective separation of organic dyes using ionic liquids as green mobile phase and polyaniline-modified silica gel nanocomposite-based thin-layer chromatography, *Journal of Analytical Science and Technology*, Issue 18, Aug **2017**, DOI 10.1186/s40543-017-0127-8

[3] IUPAC, Compendium of Chemical Terminology, 2nd ed. (the "Gold Book") (1997). "Retardation factor, RF in planar chromatography". doi:10.1351/goldbook.R05353

[4] Hattori, Hideshi & Arudra, Palani & Abdalla, Amr & Al-Khattaf, Sulaiman. (2020). Infrared Study of Silanol Groups on Dealuminated High Silica MFI Zeolite to Correlate Different Types of Silanol Groups with Activity for Conversion of 1-Butene to Propene. Catalysis Letters. 150. 10.1007/s10562-019-02972-8.

6.0 Appendix

6.1 Run Details

TLC plates	Silica Gel 60 F254 (5x10cm)
Date	10/25/2023
Time	10:10 AM to 11:20 AM

6.2 Formulas

$R_f = d_0 / d_1$ In this document d_0 is used to represent the distance the MP travels, d_1 the distance of the analyte.

e.g. $R_f = 21.5mm/80mm = 0.27$

6.3 Significant Figures

Significant figures used for intermediate values were the significant figures of the raw data plus one, for all calculation results.

6.4 Data

All raw data was expressed in the body of this document.