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# MINING FREQUENT PATTERN ON LIQUID CHROMATOGRAPHY-MASS SPECTROMETER DATA OF RODENT TUBER TO FIND THE ASSOCIATION RULES OF COMPOUNDS FOR MACHINE LEARNING

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Abstract. *Liquid Chromatography-Mass Spectrometry (LC-MS) data contains a lot of measurement data, which contains Rodent Tuber plant measurement. This paper aims to obtain frequent patterns on LCMS data utilizing the FP-Growth method, where frequent patterns will be used for interpretation and identification of chemical compound in a biological sample. For faster computation, it needed representative sampling data and linear systematic sampling was utilized for this purpose. In conclusion, FP-Growth can be applied successfully on Rodent Tuber's sample LC-MS data and generate an association rule of chemical compound formula. The result showed that C49H79O13P was the dominant compound in the LC-MS data with the highest support value of 41.67%. However, it depends on the other, namely [C43H49O23]+. It can be said that when [C43H49O23]+ appears, there is possibility that C49H79O13P will also appear. Also, there is a perfect rule that [C43H49O23]+ implies C49H79O13P but not conversely. These results can be utilized for rule-based machine learning.*

**Keywords:** LC-MS, Frequent pattern growth, Rodent Tuber, Data mining, Linear systematic sampling, Machine learning

1. **Introduction.** Raw data of Liquid Chromatography-Mass Spectrometry (LC-MS) contains millions of data points, and there are hundreds to thousands of chromatographic peaks, after integration and peak extraction [1]. This raw data provide highly complex biological samples [2]. Meanwhile, LC-MS is widely used, especially in the interpretation or identification of the content of chemical compounds in a biological sample [2-6]. It consists of hundreds of thousands of mass per charge (m/z), retention time, and intensity [7]. Likewise, the LC-MS data of Rodent Tuber from the studies of Sianipar et al. [8-12] which is used in this paper.

It is not trivial to processing this data to be useful information. Many techniques are used to process these data. It is challenging to extract the information contained in the LC-MS data. One of them is finding certain patterns in the data with association rule. This pattern can be used to find and recognize certain chemical compounds contained in other LC-MS data. Further, the association rule can be used in machine learning.

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Association rule is a standardized and well-researched technique for finding interesting relationships between variables [13], finding causalities between variables [14], finding the frequent patterns in large databases [15,16], and feature selection for classification [17]. The association rule is obtained by searching the frequent itemset of all frequent itemsets in the database. The discovery of frequent itemset is an important step in obtaining association rule [18]. Two algorithms are popular in this field, namely Apriori and FP-Growth which are mining frequent patterns from a set of transactions in horizontal data format [19], although utilize only one minimum support threshold [20].

FP-Growth was chosen because of its popularity [16], with a moderate time complexity and space complexity [21], among the best ones to extract association patterns of the frequent item sets [22], and advantages such as more efficient than Apriori and adopting divide and conquer strategy as discussed in [13,15,23-25].

This paper describes pattern mining on Rodent Tuber's LC-MS data to obtain association rules to determine the relationships and/or casualities between chemical compounds. The results of this study will help biological or pharmaceutical scientists to 1) analyze and identify the possible presence of other compounds, 2) find interesting relationships and/or casualities between compounds, 3) find the frequent patterns, and 4) can be used for further processing in machine learning.

This paper categorized as follows. Section 2 describes the problem statement and preliminaries process for further processing. Section 3 describes the research method. Section 4 describes the result and discussion. Section 5 focuses on conclusions and future work.

2. **Problem Statement and Preliminaries.** LC-MS data contain a huge number of compounds. It is possible to identify compounds that frequently occur and their association with other compounds. It can be established as a pattern for other purposes.

There are more than 700,000 records in each of LC-MS data of Rodent Tuber from studies of Sianipar et al. [8-12]. Their studies resulted in 10 datasets. We have calculated, to get one compound name manually, will take about 20 seconds at the fastest time. By a simple calculation, about 700,000 records will take 20 × 700,000 seconds. That means 5 months to complete the labeling for each dataset.

Using large native data like this case would be expensive. Therefore, data sampling will be carried out. This LC-MS data of Rodent Tuber is time-series data, so it is required sampling per period to obtain a representative sample that represents the actual dataset. Linear systematic sampling is chosen because it is simple and can represent the actual dataset.

To obtain a systematic sample, given that $N$ is the number of population of elements, while $n$ is the number of samples desired, so if $N/n$ is an integer, then $k = N/n$; otherwise, let $k$ be the next integer after $N/n$.

After that, find a random integer $R$ between 1 and $k$, defining the sample as the unit numbered $R$, $R + k$, $R + 2k$, and so on. The initial unit $R$ selected is called "random start" and $k$ is called "sampling interval" [26,27].

Based on that, we develop Algorithm 1 as shown in Figure 1 and it is implemented in python. It is applied to one of ten of the LC-MS dataset of Rodent Tuber which we have and the result will be used in this paper.

3. **Research Method.** Our research method consists of several stages as shown in Figure 2. Preliminaries above are the first stage of this method as preparation and initial stage by implementing Algorithm 1 as shown in Figure 1 utilizing linear systematic sampling. This preliminary generates a representative sample of the whole dataset. It generates 18,271 records out of 985,924 records.

```
Algorithm 1: LSS
Result: Sample File in type .xlsx
df = pd.read_excel(file)
listDF = df.values.tolist()
pivotDF = pd.pivot_table(df,index = ["Retention Time"])
arrRT = pivotDF.index.array.to_numpy()
arrSample = [ ]
arrTempRT = [ ]
arrTempI = [ ]
totalRec = 0
k = len(listDF)/18000
for n in range(len(arrRT)):
    arrTemp = [ ]
    arrTempInt = [ ]
    for i in range(len(listDF)):
        if listDF[i][2] == arrRT[n]:
            arrTemp.append(listDF[i][0])
            arrTempInt.append(listDF[i][1])
    indexes = np.arrange(random.randint(0,k),
                                    len(arrTemp), step = k)
    for i in indexes:
        arrSample.append(arrTemp[i])
        arrTempRT.append(arrRT[n])
        arrTempI.append(arrTempInt[i])
df1.to_excel('sampleFile.xlsx', index = False)
```

Figure 1. Algorithm of linear systematic sampling

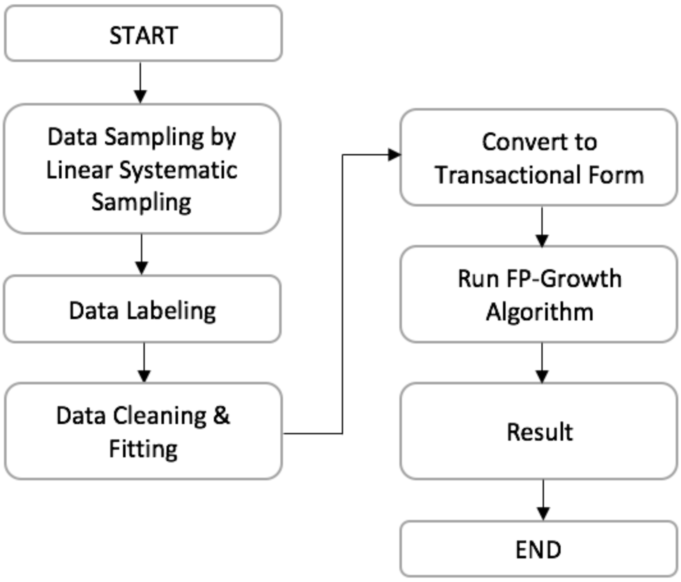
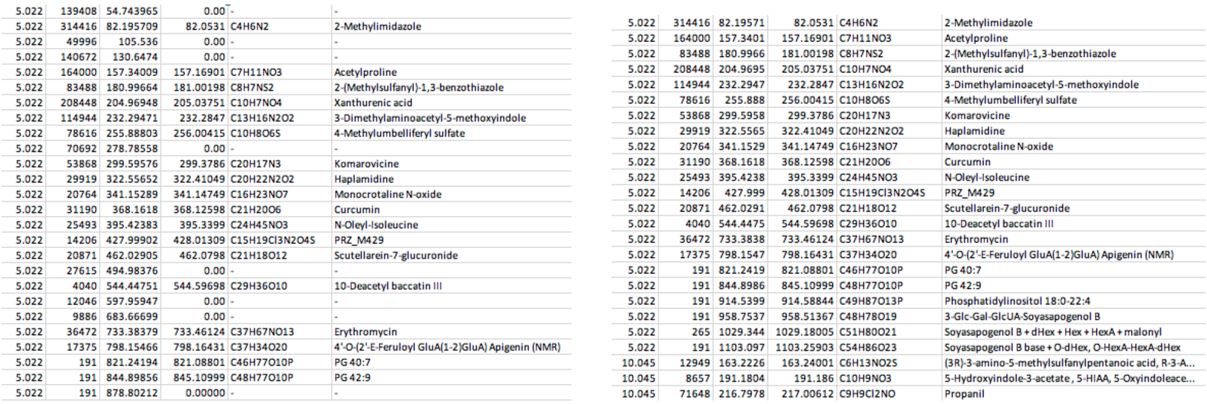


Figure 2. Diagram of research method

FP-Growth is commonly used for market basket analysis which usually has data label. The LCMS data to be analyzed does not have label, so it needs to be labeled. Labeling in this study is giving name of chemical compounds based on their mass per charge (m/z) in the data. After labeling, the LCMS data will be converted into a kind of transactional data like a market basket transaction. It is labeled with another previously developed tool in stage "Data Labeling".

The result of labeling is an unclean dataset as shown in Figure 3(a), because there are several missing names of chemical compounds; in this case, we write it with characters "-". Therefore, "Data Cleaning and Fitting" was carried out. Data cleaning and fitting is intended to dispose empty data or has no label. It generates dataset as shown in Figure 3(b).



|  |  |  |  |  |  |
|---|---|---|---|---|---|
| 5.022 | 139408 | 54.743965 | 0.00 | - | - |
| 5.022 | 314416 | 82.195709 | 82.0531 | C4H6N2 | 2-Methylimidazole |
| 5.022 | 49996 | 105.536 | 0.00 | - | - |
| 5.022 | 140672 | 130.6474 | 0.00 | - | - |
| 5.022 | 164000 | 157.34009 | 157.16901 | C7H11NO3 | Acetylproline |
| 5.022 | 83488 | 180.99664 | 181.00198 | C8H7NS2 | 2-(Methylsulfanyl)-1,3-benzothiazole |
| 5.022 | 208448 | 204.96948 | 205.03751 | C10H7NO4 | Xanthurenic acid |
| 5.022 | 114944 | 232.29471 | 232.2847 | C13H16N2O2 | 3-Dimethylaminoacetyl-5-methoxyindole |
| 5.022 | 78616 | 255.88803 | 256.00415 | C10H8O6S | 4-Methylumbelliferyl sulfate |
| 5.022 | 70692 | 278.78558 | 0.00 | - | - |
| 5.022 | 53868 | 299.59576 | 299.3786 | C20H17N3 | Komarovicine |
| 5.022 | 29919 | 322.55652 | 322.41049 | C20H22N2O2 | Haplamidine |
| 5.022 | 20764 | 341.15289 | 341.14749 | C16H23NO7 | Monocrotaline N-oxide |
| 5.022 | 31190 | 368.1618 | 368.12598 | C21H20O6 | Curcumin |
| 5.022 | 25493 | 395.42383 | 395.3399 | C24H45NO3 | N-Oleyl-Isoleucine |
| 5.022 | 14206 | 427.99902 | 428.01309 | C15H19Cl3N2O4S | PRZ_M429 |
| 5.022 | 20871 | 462.02905 | 462.0798 | C21H18O12 | Scutellarein-7-glucuronide |
| 5.022 | 27615 | 494.98376 | 0.00 | - | - |
| 5.022 | 4040 | 544.44751 | 544.59698 | C29H36O10 | 10-Deacetyl baccatin III |
| 5.022 | 12046 | 597.95947 | 0.00 | - | - |
| 5.022 | 9886 | 683.66699 | 0.00 | - | - |
| 5.022 | 36472 | 733.38379 | 733.46124 | C37H67NO13 | Erythromycin |
| 5.022 | 17375 | 798.15466 | 798.16431 | C37H34O20 | 4'-O-(2'-E-Feruloyl GluA(1-2)GluA) Apigenin (NMR) |
| 5.022 | 191 | 821.24194 | 821.08801 | C46H77O10P | PG 40:7 |
| 5.022 | 191 | 844.89856 | 845.10999 | C48H77O10P | PG 42:9 |
| 5.022 | 191 | 878.80212 | 0.00000 | - | - |

(a)

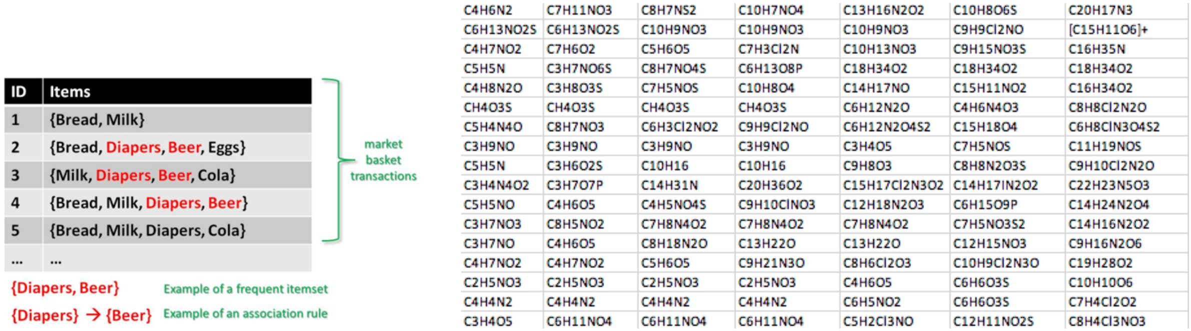
|  |  |  |  |  |  |
|---|---|---|---|---|---|
| 5.022 | 314416 | 82.19571 | 82.0531 | C4H6N2 | 2-Methylimidazole |
| 5.022 | 164000 | 157.3401 | 157.16901 | C7H11NO3 | Acetylproline |
| 5.022 | 83488 | 180.9966 | 181.00198 | C8H7NS2 | 2-(Methylsulfanyl)-1,3-benzothiazole |
| 5.022 | 208448 | 204.9695 | 205.03751 | C10H7NO4 | Xanthurenic acid |
| 5.022 | 114944 | 232.2947 | 232.2847 | C13H16N2O2 | 3-Dimethylaminoacetyl-5-methoxyindole |
| 5.022 | 78616 | 255.888 | 256.00415 | C10H8O6S | 4-Methylumbelliferyl sulfate |
| 5.022 | 53868 | 299.5958 | 299.3786 | C20H17N3 | Komarovicine |
| 5.022 | 29919 | 322.5565 | 322.41049 | C20H22N2O2 | Haplamidine |
| 5.022 | 20764 | 341.1529 | 341.14749 | C16H23NO7 | Monocrotaline N-oxide |
| 5.022 | 31190 | 368.1618 | 368.12598 | C21H20O6 | Curcumin |
| 5.022 | 25493 | 395.4238 | 395.3399 | C24H45NO3 | N-Oleyl-Isoleucine |
| 5.022 | 14206 | 427.999 | 428.01309 | C15H19Cl3N2O4S | PRZ_M429 |
| 5.022 | 20871 | 462.0291 | 462.0798 | C21H18O12 | Scutellarein-7-glucuronide |
| 5.022 | 4040 | 544.4475 | 544.59698 | C29H36O10 | 10-Deacetyl baccatin III |
| 5.022 | 36472 | 733.3838 | 733.46124 | C37H67NO13 | Erythromycin |
| 5.022 | 17375 | 798.1547 | 798.16431 | C37H34O20 | 4'-O-(2'-E-Feruloyl GluA(1-2)GluA) Apigenin (NMR) |
| 5.022 | 191 | 821.2419 | 821.08801 | C46H77O10P | PG 40:7 |
| 5.022 | 191 | 844.8986 | 845.10999 | C48H77O10P | PG 42:9 |
| 5.022 | 191 | 914.5399 | 914.58844 | C49H87O13P | Phosphatidylinositol 18:0-22:4 |
| 5.022 | 191 | 958.7537 | 958.51367 | C48H78O19 | 3-Glc-Gal-GlcUA-Soyasapogenol B |
| 5.022 | 265 | 1029.344 | 1029.18005 | C51H80O21 | Soyasapogenol B + dHex + Hex + HexA + malonyl |
| 5.022 | 191 | 1103.097 | 1103.25903 | C54H86O23 | Soyasapogenol B base + O-dHex, O-HexA-HexA-dHex |
| 10.045 | 12949 | 163.2226 | 163.24001 | C6H13NO2S | (3R)-3-amino-5-methylsulfanylpentanoic acid, R-3-A... |
| 10.045 | 8657 | 191.1804 | 191.186 | C10H9NO3 | 5-Hydroxyindole-3-acetate , 5-HIAA, 5-Oxyindoleace... |
| 10.045 | 71648 | 216.7978 | 217.00612 | C9H9Cl2NO | Propanil |

(b)

Figure 3. (a) Snippet unclean data; (b) snippet cleaning and fitting result

After the dataset is cleaned and fit according to what we want, it needs to be processed again to adjust the use of the FP-Growth algorithm. We called it "Convert to Transactional Form".

This process converts the dataset to the transactional form as a market basket transaction as illustrated in Figure 4(a) and the snippet of real result dataset of this conversion is shown in Figure 4(b). In this case, Transaction ID (TID) is Retention Time and the item set is compound formula. It is done by Algorithm 2 as seen in Figure 5. Retention Time is a TID because it has many duplicates with different m/z, as in actual case, a grocery receipt consisting of many shopping items with one TID which actually can be interpreted that there are many duplicates of TID because it is attached to one item which is then summarized into one.



| ID | Items |
|---|---|
| 1 | {Bread, Milk} |
| 2 | {Bread, Diapers, Beer, Eggs} |
| 3 | {Milk, Diapers, Beer, Cola} |
| 4 | {Bread, Milk, Diapers, Beer} |
| 5 | {Bread, Milk, Diapers, Cola} |
| ... | ... |

market basket transactions
{Diapers, Beer}
Example of a frequent itemset
{Diapers} → {Beer}
Example of an association rule

(a)

|  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|
| C4H6N2 | C7H11NO3 | C8H7NS2 | C10H7NO4 | C13H16N2O2 | C10H8O6S | C20H17N3 |
| C6H13NO2S | C6H13NO2S | C10H9NO3 | C10H9NO3 | C10H9NO3 | C9H9Cl2NO | [C15H11O6]+ |
| C4H7NO2 | C7H6O2 | C5H6O5 | C7H3Cl2N | C10H13NO3 | C9H15NO3S | C16H35N |
| C5H5N | C3H7NO6S | C8H7NO4S | C6H13O8P | C18H34O2 | C18H34O2 | C18H34O2 |
| C4H8N2O | C3H8O3S | C7H5NOS | C10H8O4 | C14H17NO | C15H11NO2 | C16H34O2 |
| CH4O3S | CH4O3S | CH4O3S | CH4O3S | C6H12N2O | C4H6N4O3 | C8H8Cl2N2O |
| C5H4N4O | C8H7NO3 | C6H3Cl2NO2 | C9H9Cl2NO | C6H12N2O4S2 | C15H18O4 | C6H8ClN3O4S2 |
| C3H9NO | C3H9NO | C3H9NO | C3H9NO | C3H4O5 | C7H5NOS | C11H19NOS |
| C5H5N | C3H6O2S | C10H16 | C10H16 | C9H8O3 | C8H8N2O3S | C9H10Cl2N2O |
| C3H4N4O2 | C3H7O7P | C14H31N | C20H36O2 | C15H17Cl2N3O2 | C14H17IN2O2 | C22H23N5O3 |
| C5H5NO | C4H6O5 | C4H5NO4S | C9H10ClNO3 | C12H18N2O3 | C6H15O9P | C14H24N2O4 |
| C3H7NO3 | C8H5NO2 | C7H8N4O2 | C7H8N4O2 | C7H8N4O2 | C7H5NO3S2 | C14H16N2O2 |
| C3H7NO | C4H6O5 | C8H18N2O | C13H22O | C13H22O | C12H15NO3 | C9H16N2O6 |
| C4H7NO2 | C4H7NO2 | C5H6O5 | C9H21N3O | C8H6Cl2O3 | C10H9Cl2N3O | C19H28O2 |
| C2H5NO3 | C2H5NO3 | C2H5NO3 | C2H5NO3 | C4H6O5 | C6H6O3S | C10H10O6 |
| C4H4N2 | C4H4N2 | C4H4N2 | C4H4N2 | C6H5NO2 | C6H6O3S | C7H4Cl2O2 |
| C3H4O5 | C6H11NO4 | C6H11NO4 | C6H11NO4 | C5H2Cl3NO | C12H11NO2S | C8H4Cl3NO3 |

(b)

Figure 4. (a) Illustration of market basket transactions [28]; (b) snippet data

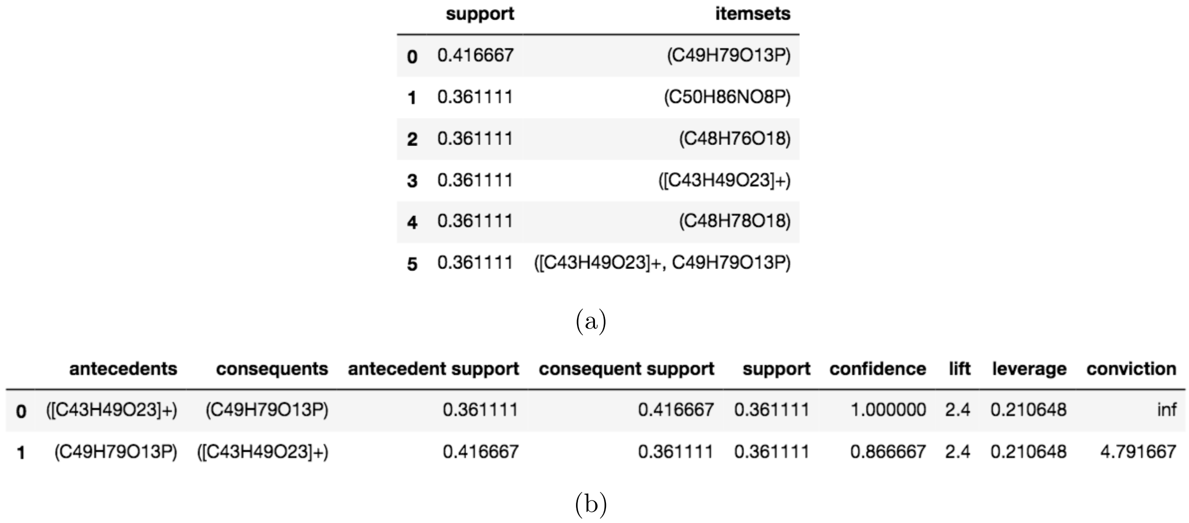
The next step is "Run FP-Growth Algorithm" which is processing the transactional form as a market basket transaction into FP-Growth algorithm. This aims to find the frequent pattern of item. This algorithm is implemented in the python library namely mlxtend.frequent_patterns [29]. We just used it without adjustment of anything.

```
Algorithm 2: Convert to market basket transaction
1: Get data from the dataset that has been made into an excel file and convert it to
   an actual data list, namely listDF[ ].
2: Create a list that contains "Retention Time" column without duplication with the
   pivot feature, namely arrRT[ ].
3: rows, cols = (len(arrRT), len(listDF))
   arr = [ ]
4: for $i$ in range(rows):
         row = [ ]
         for $j$ in range (cols):
               if listDF[$j$][4] == arrRT[$i$]:  ⇒ check same Retention Time
                     row.append(listDF[$j$][1])  ⇒ append Formula to new list as row
5:       arr.append(row)  ⇒ append list ROW in list ARR
   Write arr[ ] list into an excel file.  ⇒ result is 2D list
```

Figure 5. Algorithm of conversion to market basket transaction

4. **Result and Discussion.** Linear systematic sampling successfully builds a sample of LC-MS data which at first contains 985,924 records to 18,271 records done by Algorithm 1. Using a smaller number of data sample but representing the entire dataset, labeling work is faster. Labeling was done by web scraping technique [30-32]. The next steps are cleaning and fitting, converting, and processed to FP-Growth algorithm. All processes are implemented in python programming and FP-growth library.

The results of FP-Growth processing with minimum support of 35% are shown in Figure 6(a) and the association rule with a minimum threshold of 35% is shown in Figure 6(b).



| | support | itemsets |
|---|---|---|
| 0 | 0.416667 | (C49H79O13P) |
| 1 | 0.361111 | (C50H86NO8P) |
| 2 | 0.361111 | (C48H76O18) |
| 3 | 0.361111 | ([C43H49O23]+) |
| 4 | 0.361111 | (C48H78O18) |
| 5 | 0.361111 | ([C43H49O23]+, C49H79O13P) |

(a)

| | antecedents | consequents | antecedent support | consequent support | support | confidence | lift | leverage | conviction |
|---|---|---|---|---|---|---|---|---|---|
| 0 | ([C43H49O23]+) | (C49H79O13P) | 0.361111 | 0.416667 | 0.361111 | 1.000000 | 2.4 | 0.210648 | inf |
| 1 | (C49H79O13P) | ([C43H49O23]+) | 0.416667 | 0.361111 | 0.361111 | 0.866667 | 2.4 | 0.210648 | 4.791667 |

(b)

Figure 6. (a) Result of FP-Growth; (b) result of association rule

As seen in Figure 6(a), the highest support is 0.416667 and it is a single item, which is C49H79O13P, namely Phosphatidylinositol 20:4-20:4 with exact mass is 906.52582; it means this item appears most frequently (41.6%) in the data. It is dominant compared with others.

In Figure 6(b), the highest confidence value is 1.00, this can be interpreted that if there is [C43H49O23]+, then absolutey C49H79O13P exists, but if conversely, the confidence value is smaller (86.67%) which is still a good value because of more than 50%. This result has an association with lift value which is more than 1. They are dependent on each other as can be seen in the leverage value which is more than 0.

The conviction will be infinity ($\infty$) if a perfect rule and has a value of 1 if it is a completely uncorrelated rule [33]. [C43H49O23]+ implies C49H79O13P has conviction infinity which means that this is a perfect rule of the antecedent ([C43H49O23]+) to the consequent (C49H79O13P) but not conversely.

5. **Conclusion.** The FP-Growth algorithm using python library namely mlxtend. frequent_patterns can be applied well to the sample of LC-MS data of Rodent Tuber. Sampling was done by the linear systematic sampling method. It serves to simplify a huge dataset but still reflects the actual dataset.

There is a huge number of compounds in LC-MS data of Rodent Tuber when closely observed. However, by processing the dataset with FP-Growth, it can be seen which compounds are more dominant than others. As seen from Figure 6(a), the highest support value is 41.67% which is C49H79O13P. It can be interpreted that C49H79O13P is the dominant compound in this LC-MS data. However, this compound depends on other compounds, namely [C43H49O23]+ which can be seen in Figure 6(b) with an infinity conviction value. It can also be said that when [C43H49O23]+ appears, absolutely C49H79O13P will also appear. These association rules can be utilized for processing with rule-based machine learning.

For future works, this study will be applied to other datasets from the existing LC-MS data of Rodent Tuber so that more comprehensive results are obtained.

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