ABSTRACT
Qualitative and quantitative determination of chemicals especially that deal with drugs and pharmaceuticals can be considered as important issues in scientific research to insure human safety and security. And with this direction of scientific research, saccharin as sodium salt had been determined qualitatively and quantitatively through applying FTIR technique. By applying a weight range of the pure compound, the obtained results showed several absorption bands with standard curves that obeyed Lambert-Beer Law. Also, HPLC technique applied and compared with FTIR of different pharmaceuticals in local Iraqi market. The %E and Rec., % results for both methods proved that sampling procedure in FTIR technique for both pure and pharmaceutical samples was more superiority beside simplicity.

Keywords: Saccharin, FTIR, HPLC, sweeteners, quantitative determination.

INTRODUCTION
Sweeteners counted as cheap food additives that several times duplicated sugar taste compared with natural sugar (sucrose) with other benefits such as decreasing the resulted energy from food consumption that afforded to weight loss (diet), solving sugar or insulin regulation problems in Diabetes mellitus and Hyperglycemia respectively, and teeth protection. Sweeteners can be classified according to their source to Natural sugar substitutes (natural sweeteners) [Brazzein, Curculin, Mabinlin, Miraculin, Monellin, Pentadin, Thaumatin, Erthritol, Glycyrrhizin, Glycerol, hydrogenated starch hydrolysates, Inulin …etc.] (Figure 1) obtained from plants such as proteins, polyols, monosaccharide, extracts with sweetness by weight ranged from 0.4 to 3000

The other classification of sweeteners is Artificial sweeteners or sugar substitutes [Alietame, Aspartame, Dulcin, Glucin, Neotame, P-4000, Saccharin sodium, …. etc.] (Figure 2) with sweetness by weight range (30-8000) as a free or $K^+$, $Na^+$, or $Ca^{2+}$ salt prepared with different methods according to its chemical structure.

Saccharin as sodium salt: 2 – Sodio - 1, 2 – benzisothiazol - 3(2$H$) - one 1, 1 - dioxide (Figure 3) is artificial sweeteners applied in several food industries (Teeth pastes, soft drink, candies, and others) and in production of several pharmaceuticals as low calories sweet material. Saccharin sweetness estimated from 300 to 500 times than sucrose and its known as a crystalline white powder with different water contain, soluble in water, slightly soluble in alcohol with different scientific names.

*Chem. Abstr. Name: 1,2-Benzisothiazol-3(2$H$)-one, 1,1-dioxide, sodium salt
IUPAC Systematic Name: 1,2-Benzisothiazolin-3-one, 1,1-dioxide, sodium salt
Synonyms: ortho-Benzozylsulfimide sodium salt; saccharin sodium; saccharin sodium salt; saccharin soluble; sodium ortho-benzosulfimide; sodium
saccharide; sodium saccharinate; sodium saccharine; soluble saccharin
Saccharin can be prepared as heterocycles according to Remens-Fahlberg synthesis\(^1\) (Scheme -1-) or Maumee synthesis\(^1\) (Scheme -2-).
Saccharin does not digest in human and does not help in insulin production as a result of taste\(^5\). Also, World Health Organization (WHO) through International Agency for Research on Cancer (IARC) classified it as Group 2B, possibly carcinogenic to humans then changed it to Group 3, not classifiable as to carcinogenicity to humans. This WHO classification was documented in spite of numerous scientific research pointed to saccharin carcinogenicity on rats, mice, monkeys (especially balder cancer in rats) because WHO considered this material as non-DNA-reactive mechanism\(^5\).

Despite of the negative effect produced as a result of long term consumption, increasing of world usage and production had been distributed between human personal care, industrial or pharmaceutical products. This matter cleared international scientific research institutes ways to document the suitable analytical methods to determine saccharin sodium such as classical (titration), colorimetric, spectroscopic, electrical, chromatographic methods\(^9\)\(^-\)\(^21\).
The aim of this work was directed to use FTIR technique for qualitative and quantitative determination of saccharin sodium.

EXPERIMENTAL

Chemicals

All used chemicals were from BDH and were used without further purification. Tablets samples were from local Iraqi market with WHO note of allowed sodium cyclamate (0-12)mg/Kg human weight and sodium saccharin (2.5-5)mg/Kg.

- Sample (A): Dulcaryl from BİLİM PHARMACEUTICALS containing Sodium cyclamate (31.250)mg, Sodium saccharin 2H\(_2\)O (3.125)mg
- Sample (B): Sweetcell from MEDCELLPHARMA containing Sweetener: sodium cyclamate, sodium saccharin, lactose

Acidity regulators: sodium hydrogen carbonate, monosodium citrate where sweetness equal to 4.4 gm of sugar.

For standard curve with FTIR technique, (0.4-2.5) mg of standard saccharin sodium was applied. Ten tablets from commercial sweetener were weighted and grinded then calculated for one tablet as an average weight. A weight of the resulted powder equaled to 0.100 mg of pure saccharin sodium was mixed with pure KBr powder.

Instrument

FTIR spectra were recorded using KBr discs on Shimadzu (Japan) IRPestige-21 / FTIR-8000 Fourier Transform Infrared spectrophotometer.

HPLC method was achieved with CeCell instrument with below analytical conditions:

- Mobile phase: 50:50 water: methanol with 2 drops of trifluoracetic acid (TFA).
- Column: C18, 25 cm, 4.6 mm i.d x 5μL, particle size 3μm.
- Temperature: room temperature.
- Flow rate: 0.7 mL / min.
- Detector: U.V at 220 nm
- Volume of Injection: 50 μL

RESULTS AND DISCUSSION

FTIR spectrum of pure saccharin sodium showed several absorption bands coincided with literatures\(^22\)\(^-\)\(^27\) such as C=O absorption at 1643 cm\(^-1\), C-C benzene ring stretching at (1585, 1458) cm\(^-1\), -SO\(_2\)-N-stretching at (1336, 1257, 1149) cm\(^-1\) including asymmetrical and symmetrical -SO\(_2\)- stretching vibrations. Negative Saccharinate ion with asymmetrical absorption and carbonyl bending were appeared at (968 and 748) cm\(^-1\) respectively.

The resulted FTIR spectra (Figure 4) of accurate weighted pure saccharin sodium gave several standard equations (Table 1) according to different wave numbers with identical R\(^2\) results. Table 2 showed the efficiency of the applied FTIR method for quantitative determination of saccharin sodium in commercial samples (Figure 5).

The choosing of the proper wave number was based on its representation of special group in saccharin sodium structure did not repeat in other components presented in commercial tablet beside its symmetry with increasing weight with high R\(^2\) value. On the previous bases, 1643 cm\(^-1\) had been selected.

Many literatures\(^28\)\(^-\)\(^33\) came out HPLC technique applications in qualitative and/or quantitative determination of saccharin sodium in many different samples and analytical conditions. In this work, HPLC technique had been applied as reference method to determine saccharin sodium where the obtained resulted with range (0.8-4) ppm of pure saccharin sodium gave good standard curve results (R\(^2\)=0.995) (Figures-6- and -7-) had been used for comparison with FTIR technique results. In HPLC technique, one commercial tablet dissolved in one litre of deionized water (Figure 8).

Tabulated E% and Rec.% results (Table 2 and Table 3) showed that the Turkish sample gave approach values in both applied methods and FTIR method was outdoing than HPLC method in approaching to the recorded values by the manufacture. The final result
for this work with FTIR technique proved that this new quantitative saccharin sodium determination method is with high efficiency compared with the classic HPLC or other analytical method.

CONCLUSIONS
FTIR technique provided with its simplicity in sampling procedure for both pure and commercial samples compared with others. FTIR simplicity and accuracy were obvious in our obtained results in determination saccharin sodium.

<table>
<thead>
<tr>
<th>Wave number, cm⁻¹</th>
<th>Standard curve equation</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1643</td>
<td>y = 1.177x + 0.988</td>
<td>0.973</td>
</tr>
<tr>
<td>1585</td>
<td>y = 0.137x + 0.075</td>
<td>0.985</td>
</tr>
<tr>
<td>1458</td>
<td>y = 0.084x + 0.099</td>
<td>0.992</td>
</tr>
<tr>
<td>1336</td>
<td>y = 0.133x + 0.065</td>
<td>0.993</td>
</tr>
<tr>
<td>1149</td>
<td>y = 0.280x - 0.018</td>
<td>0.953</td>
</tr>
<tr>
<td>968</td>
<td>y = 0.184x + 0.033</td>
<td>0.975</td>
</tr>
<tr>
<td>748</td>
<td>y = 0.170x + 0.024</td>
<td>0.975</td>
</tr>
</tbody>
</table>

Table 2
Results of applied FTIR method.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Tablet weight, mg</th>
<th>FTIR results at 1643 cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average of one tablet</td>
<td>Applied</td>
</tr>
<tr>
<td>A</td>
<td>42.6</td>
<td>1.125</td>
</tr>
<tr>
<td>B</td>
<td>55.9</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Table 3
Results of applied HPLC method.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>One tablet weight, mg</th>
<th>HPLC results at 4.17 min. as a retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Resulted sodium saccharin, mg</td>
</tr>
<tr>
<td>A</td>
<td>42.6</td>
<td>4.58907</td>
</tr>
<tr>
<td>B</td>
<td>55.9</td>
<td>6.739814</td>
</tr>
</tbody>
</table>
Figure 1
Chemical structures of several natural sweeteners.

Figure 2
Chemical structures of several artificial sweeteners or sugar substitutes.
Figure 3
Saccharin and its salts chemical structures.

Scheme 1
Remsen-Fahlberg synthesis.

Scheme 2
Maumee synthesis.
Figure 4
FTIR spectra for saccharin sodium with different weights (0.4-2.5) mg.

Figure 5
FTIR spectral for two commercial sweeteners containing saccharin sodium.
Figure 6
HPLC chromatogram for pure saccharin sodium (3.2 and 4) ppm

Figure 7
HPLC standard curve for saccharin sodium.

Figure 8
HPLC chromatogram for two commercial sweeteners (Turkish-made (A) and Dutch-made (B)) samples

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REFERENCES


