Valproic Acid and Sodium Valproate: Comprehensive Profile

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   6.3. Metabolism
   6.4. Excretion
   6.5. Distribution and protein binding

References
1.1.3. **Proprietary names [2–4]**

Epival®, Epicon®, Epilin® (Sanofi Winthrop), Ergenyl® (Sanofi Winthrop), Convulex® (Pharmacia & Upjohn), Dépakin® (Sanofi Winthrop), Depamide®, Depa®, Depakene® (Abbott), Depakin® (Sanofi Winthrop), Depakine® (Sanofi Winthrop), Depakote®, Depakine®, Deproic®, Leptilan® (Novartis), Mylproin® (Desitin), Orfilept® (Leo), Orfril® (Desitin), Orlept® (CP Pharmaceuticals), Valcote®, Vistora®.

1.1.4. **Synonyms [3]**

Valproic acid
Abbott-44089
Sodium valproate
Abbott-44090

1.2. **Formulae**

1.2.1. **Empirical formula, molecular weight, and CAS number [3,4]**

<table>
<thead>
<tr>
<th>Form</th>
<th>Empirical formula</th>
<th>Molecular weight</th>
<th>CAS number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid form</td>
<td>C₈H₁₆O₂</td>
<td>144.2</td>
<td>99-66-1</td>
</tr>
<tr>
<td>Sodium salt</td>
<td>C₈H₁₅NaO₂</td>
<td>166.5</td>
<td>1069-66-5</td>
</tr>
</tbody>
</table>

1.2.2. **Structural formulae**

- Valproic acid
  \[
  H₂C₆H₆CH₂ \xrightarrow{\text{CHCOOH}}
  \]
- Sodium valproate
  \[
  H₂C₆H₆CH₂ \xrightarrow{\text{CHCOONa}}
  \]

1.3. **Elemental analysis**

The calculated elemental composition is as follows:

<table>
<thead>
<tr>
<th>Element</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>66.63%</td>
</tr>
<tr>
<td>H</td>
<td>11.18%</td>
</tr>
<tr>
<td>O</td>
<td>22.19%</td>
</tr>
</tbody>
</table>

1.4. **Appearance [1,3]**

**Acid form**

Valproic acid is a colorless to pale yellow, slightly viscous, and clear liquid having a characteristic odor.
Salt form
Sodium valproate is a white or almost white, hygroscopic, crystalline, and deliquescent powder.

2. PHYSICAL CHARACTERISTICS

2.1. Properties

Colorless liquid with characteristic odor. Boiling point 219.5 °C. $n_D^{24.5} = 1.425$. $d_1^0 = 0.9215$. $pK_a = 4.6$. LD$_{50}$ orally in rats 670 mg/kg [2].

2.2. Solution pH

The USP solutions of valproic acid and sodium valproate, which are prepared with the aid of sodium hydroxide, have a pH of 7–8 [3].

2.3. Solubility characteristics

Valproic acid is slightly soluble in water. It is freely soluble in acetone, alcohol, chloroform, ether, benzene, $n$-heptane, methyl alcohol, and 0.1 N sodium hydroxide; slightly soluble in 0.1 N hydrochloric acid [2,3].

Sodium valproate is very soluble in water, slight to freely soluble in alcohol [2,3].

2.4. Optical activity

Valproic acid and sodium valproate have no optical activity.

2.5. X-ray powder diffraction pattern

The X-ray powder diffraction pattern of valproic acid was performed using a Simons XRD–5000 diffractometer. Table 1 shows the values of the scattering angles (degrees 2θ), the interplanar $d$-spacings (Å), and the relative intensities (%) for valproic acid, which were automatically obtained on a digital printer. Figure 1 shows the X-ray powder diffraction pattern of valproic acid, which was carried on a pure sample of the drug.

2.6. Thermal methods of analysis

2.6.1. Melting point

Sodium valproate does not melt, decompose, or physically change in the normal working range of the Thomas–Hoover capillary melting point apparatus [5]. Valproic acid boils at 120–121 °C [2].

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Table 1. Crystallographic data from the X-ray powder diffraction pattern of sodium valproate

<table>
<thead>
<tr>
<th>Scattering angle (degrees 2θ)</th>
<th>d-spacing (Å)</th>
<th>Relative intensity (%)</th>
<th>Scattering angle (degrees 2θ)</th>
<th>d-spacing (Å)</th>
<th>Relative intensity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.633</td>
<td>15.6762</td>
<td>42.80</td>
<td>6.088</td>
<td>14.5046</td>
<td>4.85</td>
</tr>
<tr>
<td>6.691</td>
<td>13.1992</td>
<td>100.00</td>
<td>7.319</td>
<td>12.0678</td>
<td>9.72</td>
</tr>
<tr>
<td>11.293</td>
<td>7.8290</td>
<td>9.61</td>
<td>12.178</td>
<td>7.2620</td>
<td>4.25</td>
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<tr>
<td>16.907</td>
<td>5.2397</td>
<td>38.40</td>
<td>18.177</td>
<td>4.8765</td>
<td>18.85</td>
</tr>
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<td>20.984</td>
<td>4.2299</td>
<td>43.92</td>
<td>21.772</td>
<td>4.0787</td>
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<tr>
<td>22.519</td>
<td>3.9450</td>
<td>15.40</td>
<td>24.120</td>
<td>3.6867</td>
<td>14.83</td>
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<td>24.414</td>
<td>3.6430</td>
<td>33.88</td>
<td>25.112</td>
<td>3.5433</td>
<td>4.54</td>
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<td>29.473</td>
<td>3.0281</td>
<td>7.53</td>
<td>30.022</td>
<td>2.9740</td>
<td>5.74</td>
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<td>30.881</td>
<td>2.8932</td>
<td>12.71</td>
<td>31.626</td>
<td>2.8268</td>
<td>9.03</td>
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<td>31.920</td>
<td>2.8013</td>
<td>12.25</td>
<td>32.462</td>
<td>2.7558</td>
<td>4.69</td>
</tr>
<tr>
<td>33.565</td>
<td>2.6677</td>
<td>6.05</td>
<td>34.224</td>
<td>2.6168</td>
<td>5.81</td>
</tr>
<tr>
<td>34.620</td>
<td>2.5888</td>
<td>3.81</td>
<td>36.396</td>
<td>2.4665</td>
<td>4.54</td>
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<td>36.999</td>
<td>2.4277</td>
<td>8.87</td>
<td>37.491</td>
<td>2.3969</td>
<td>5.10</td>
</tr>
<tr>
<td>37.797</td>
<td>2.3782</td>
<td>5.61</td>
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<td>2.2364</td>
<td>5.81</td>
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<td>41.110</td>
<td>2.1939</td>
<td>3.60</td>
<td>44.367</td>
<td>2.0401</td>
<td>25.37</td>
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<tr>
<td>44.848</td>
<td>2.0193</td>
<td>2.56</td>
<td>45.817</td>
<td>1.9788</td>
<td>3.36</td>
</tr>
<tr>
<td>47.086</td>
<td>1.9284</td>
<td>3.84</td>
<td>48.781</td>
<td>1.8653</td>
<td>4.14</td>
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<td>50.101</td>
<td>1.8192</td>
<td>4.50</td>
<td>51.052</td>
<td>1.7875</td>
<td>2.32</td>
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<tr>
<td>52.238</td>
<td>1.7497</td>
<td>1.20</td>
<td>53.815</td>
<td>1.7021</td>
<td>2.78</td>
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<tr>
<td>55.227</td>
<td>1.6619</td>
<td>1.84</td>
<td>55.782</td>
<td>1.6466</td>
<td>0.91</td>
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<tr>
<td>64.744</td>
<td>1.4387</td>
<td>1.48</td>
<td>77.926</td>
<td>1.2250</td>
<td>1.63</td>
</tr>
</tbody>
</table>

Fig. 1. X-ray powder diffraction pattern of sodium valproate.
2.6.2. Differential scanning calorimetry

Measurements of differential scanning calorimetry (DSC) were obtained on a TA Instruments 2910 thermal analysis system (Fig. 2). Samples of approximately 1–2 mg were accurately weighed into an aluminum DSC pan, and covered with an aluminum lid that was crimped in place. The samples were then heated over the range of 20–140 °C, at a heating rate of 10 °C/min. Valproic acid was found to boil at 227 °C.

2.7. Spectroscopy

2.7.1. Ultraviolet spectroscopy

The UV spectrum of valproic acid (0.1% solution) in methanol is shown in Fig. 3, was recorded using a Shimadzu UV–Visible Spectrophotometer 160 PC. The acid form of the drug has one maximum at 212 nm. In contrast, the sodium salt of the compound has no UV maximum between 800 and 205 nm, this in agreement with previous results reported by Chang [5].

<table>
<thead>
<tr>
<th>λ_{max} (nm)</th>
<th>A (1%, 1 cm)</th>
<th>Molar absorptivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>212</td>
<td>732</td>
<td>105.6</td>
</tr>
</tbody>
</table>

2.7.2. Vibrational spectroscopy

The infrared absorption spectrum of sodium valproate and valproic acid were obtained in a KBr disc using a Perkin-Elmer infrared spectrophotometer. The infrared spectra of sodium valproate and valproic acid are shown in Figs. 4A and
B, respectively. The infrared absorption bands assignments for sodium valproate and valproic acids are shown in Table 2(A) and (B), respectively.

2.7.3. Nuclear magnetic resonance spectrometry

2.7.3.1. $^1H$ NMR spectrum

The proton nuclear magnetic resonance (NMR) spectrum of valproic acid was obtained using a Bruker Avance Instrument operating at 300, 400, and 500 MHz. Standard Bruker Software was used to obtain COSY and HETCOR spectra. The
sample was dissolved in D$_2$O and tetramethylsilane (TMS) was used as the internal standard. The proton NMR spectrum is shown in Figs. 5 and 6. The COSY $^1$H-NMR spectra are shown in Figs. 7 and 8 and the gradient HMQC NMR spectrum is shown in Fig. 9. The assignments for the $^1$H NMR spectral of valproic acid are shown in Table 3.

Fig. 4. (A) The infrared absorption spectrum of sodium valproate obtained in a KBr pellet. (B) The infrared absorption spectrum of valproic acid obtained in a KBr pellet.
Table 2.

A. Assignments for the infrared absorption bands of sodium valproate

<table>
<thead>
<tr>
<th>Frequency (cm(^{-1}))</th>
<th>Assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>2960</td>
<td>Aliphatic C–H stretch</td>
</tr>
<tr>
<td>2930</td>
<td></td>
</tr>
<tr>
<td>2870</td>
<td></td>
</tr>
<tr>
<td>1565</td>
<td>Antisymmetrical and symmetrical stretching vibration of COO(^{-}) group</td>
</tr>
<tr>
<td>1555</td>
<td></td>
</tr>
<tr>
<td>1465</td>
<td></td>
</tr>
<tr>
<td>1415</td>
<td></td>
</tr>
</tbody>
</table>

B. Assignments for the infrared absorption bands of sodium valproate

<table>
<thead>
<tr>
<th>Frequency (cm(^{-1}))</th>
<th>Assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>3435</td>
<td>O–H stretching vibration of carboxylic acid</td>
</tr>
<tr>
<td>3065</td>
<td>Aliphatic C–H stretch</td>
</tr>
<tr>
<td>2875</td>
<td></td>
</tr>
<tr>
<td>1705</td>
<td>C=O stretch</td>
</tr>
<tr>
<td>1080</td>
<td>O–H bending vibration</td>
</tr>
</tbody>
</table>

Fig. 5. The \(^1\)H NMR spectrum of sodium valproate in D\(_2\)O.
2.7.3.2. $^{13}$C NMR spectrum

The $^{13}$C NMR spectrum of valproic acid was obtained using a Bruker Avance Instrument operating at 75, 100, and 125 MHz. Standard Bruker Software was used to obtain DEPT spectra. The sample was dissolved in D$_2$O and tetramethylsilane (TMS) was used as the internal standard. The $^{13}$C NMR spectrum of valproic acid is shown in Fig. 10. The DEPT NMR spectra are shown in Figs. 11 and 12. The assignments for the various carbons of valproic acid are presented in Table 4.

2.8. Mass spectrometry

The mass spectrum of valproic acid was obtained using a Shimadzu PQ–5000 mass spectrometer. The parent ion was collided with helium gas a carrier gas. Figure 13 shows the detailed mass fragmentation pattern. Table 5 shows the proposed mass fragmentation pattern of valproic acid.
3. STORAGE AND STABILITY

Valproic acid was found to be stable at room temperature [6]. The refrigeration or freezing of a supernatant, from blood samples containing the drug, for 7 days did not alter the total concentration of valproic acid [6,7]. Valproic acid should be stored in airtight glass, stainless steel, or polyethylene containers [3]. Valproic acid and sodium valproate should be stored in tight containers at a temperature less than 40°C, preferably between 15 and 30°C; freezing should be avoided [8]. Tablets and capsule should not be crushed [9].

4. COMPENDIAL METHODS OF ANALYSIS

4.1. Identification methods for the drug substance

4.1.1. Test A

Compare the refractive index of the substance to be examined with the refractive index of the reference standard of valproic acid, which has a known refractive index of 1.422–1.425 [10,11].
4.1.2. Test B

Examine the test article by infrared absorption spectrophotometry, and compare with the spectrum obtained with a valproic acid reference standard [10,11].

4.1.3. Test C [10,11]

Examine by thin-layer chromatography (TLC) using a suitable TLC silica gel as the coating substance on the TLC plate. The method requires preparation of the following solutions:

**Test solution.** Dissolve 50 mg of the substance to be examined in methanol and dilute to 5 mL with the same solvent.

**Reference solution.** Dissolve 50 mg of valproic acid reference standard in methanol and dilute to 5.0 mL with the same solvent.

Apply separately to the plate 2.0 μL of each solution, and develop over a path of 15 cm using a mixture of equal volumes of ether and methylene chloride. Allow the plate to dry in air and spray with bromocresol green solution. The principal spot in

Fig. 8. COSY $^1$H NMR spectrum of sodium valproate in D$_2$O.
the chromatogram obtained with the test solution is similar in position, color, and size to the principal spot in the chromatogram obtained with the reference solution.

4.1.4. Test D
To 1.0 mL of the test solution, add 3.0 mL of dilute sodium hydroxide solution and 3.0 mL of water and 1.0 mL of a 100 g/L solution of cobalt nitrate. The test is positive if violet precipitate is formed. The precipitate dissolves in methylene chloride [10,11].

4.2. Identification methods for formulated valproic acid
The United States Pharmacopoeia [12] describes two tests for the identification of valproic acid in its formulations (capsules or syrup).

4.2.1. Test A
The retention time ratio of the valproic acid peak to the internal standard peak is obtained from the standard preparation and the assay preparations.
Table 3. Proton nuclear magnetic resonance assignments for spectrum of sodium valproate

<table>
<thead>
<tr>
<th>Assignment (proton at carbon #)</th>
<th>Chemical shift, δ, (ppm relative to TMS)</th>
<th>Multiplicity (J = Hz) t: triplet, q: quartet, m: multiplet</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2.01</td>
<td>tt (5.0, 5.0)</td>
</tr>
<tr>
<td>3</td>
<td>1.16</td>
<td>m</td>
</tr>
<tr>
<td>4</td>
<td>1.02</td>
<td>tq (7.5, 7.5)</td>
</tr>
<tr>
<td>5</td>
<td>0.64</td>
<td>t (7.5)</td>
</tr>
</tbody>
</table>

Fig. 10. $^{13}$C NMR spectrum of sodium valproate in D$_2$O.

4.2.2. Test B

Place a portion of capsules contents or place a volume of syrup, equivalent to about 250 mg of valproic acid, in a separator. Add 20 mL of 1.0 M sodium hydroxide shake and allow the layers to separate. Transfer the aqueous layer to a second separator, add 4.0 mL of hydrochloric acid, mix, and extract with 40 mL $n$-heptane. Filter the $n$-heptane layer through glass wool into a beaker, and evaporate the
solvent completely on a steam bath with the aid of a current air. Transfer two drops of the residue to a test tube containing 0.5 µL each of KI (1 in 5) solution and KIO₃ (1 in 25) and mix, a yellow color is predicted.

4.3. Methods for impurities and related substances

The European Pharmacopoeia [11] contains methods to determine ten impurities in valproic acid and sodium valproate:

![Chemical structures of impurities](image)

**Fig. 11.** DEPT 45 $^1$H NMR spectrum of sodium valproate in D₂O.
Fig. 12. DEPT 45 $^1$H NMR spectrum of sodium valproate in D$_2$O.
The related substances are determined by gas chromatography (GC) using butyric acid as the internal standard, and the method requires preparation of the following three solutions.

**Internal standard solution.** Dissolve 10 mg of butyric acid in heptane and dilute to 200 mL with the same solvent.

### Table 4. $^{13}$C NMR assignments for sodium valproate

<table>
<thead>
<tr>
<th>Assignments at carbon number</th>
<th>Chemical shift, $\delta$ (ppm relative to TMS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>186.7</td>
</tr>
<tr>
<td>2</td>
<td>48.7</td>
</tr>
<tr>
<td>3</td>
<td>35.1</td>
</tr>
<tr>
<td>4</td>
<td>20.5</td>
</tr>
<tr>
<td>5</td>
<td>13.3</td>
</tr>
</tbody>
</table>

### Fig. 13. Mass spectrum of sodium valproate.

### Table 5. Assignments for the fragmentation pattern observed in the mass spectrum of sodium valproate

<table>
<thead>
<tr>
<th>$m/z$</th>
<th>Fragment</th>
</tr>
</thead>
<tbody>
<tr>
<td>145</td>
<td>$M + H^+$ mass peak ($M^+$)</td>
</tr>
<tr>
<td>126</td>
<td>$C_4H_5O_2$</td>
</tr>
<tr>
<td>102</td>
<td>$C_5H_9O_2$</td>
</tr>
<tr>
<td>73</td>
<td>$C_3H_3O_2$</td>
</tr>
</tbody>
</table>
Test solution. Dissolve 0.25 g of the substance to be examined in the internal standard solution and dilute to 5.0 mL with the same solvent. Dilute 1.0 mL of the solution to 10 mL with heptane.

Reference solution. Dissolve 20 mg of substance to be examined and 20 mg of 2-(1-methylethyl)pentanoic acid in heptane and dilute to 10 mL with the same solvent. Dilute 1.0 mL of the solution to 10 mL with heptane.

The chromatographic procedure [11] is carried out using a wide-bore fused-silica column 30 m long and 0.53 mm in internal diameter coated with macrogel 20 000 2-nitrotere-phthalate with film thickness of 0.5 μm. In addition, for chromatography, helium should be used as the carrier gas at a flow rate of 8.0 mL/min using a flame ionization detector.

The procedure is to inject 1.0 μL of each solution. The test is not valid unless, in the chromatogram obtained with reference solution, the resolution between the peaks corresponding to 2-(1-methylethyl) pentanoic acid and valproic acid is at least 2.0. In the chromatogram obtained with the test solution: the sum of the areas of the peaks, apart from the principal peak, is not greater than three times the area of the peak due to the internal standard (3.0%); none of the peaks, apart from the principal peak, has an area greater than that of the peak due to the internal standard (0.1%). Disregard any peak with an area less than 0.1 times that of the peak due to the internal standard.

4.4. Official methods of analysis

4.4.1. Raw material

The United States Pharmacopoeia [12] recommends the following assay method for valproic acid. Dissolve about 160 mg of valproic acid, accurately weighed, in 100 mL of acetone. Titrate the solution with tetrabutylammonium hydroxide titrant, taking precautions against the absorption of atmospheric carbon dioxide, determining the endpoint potentiometrically, using a glass electrode and a calomel electrode containing 0.1 M tetrabutylammonium chloride (aqueous). Perform a blank determination, and make any necessary correction. Each milliliter of 0.1 M tetrabutylammonium hydroxide titrant is equivalent to 14.42 mg of C8H16O2.

The British Pharmacopoeia [10] and the European Pharmacopoeia [11] also recommend a potentiometric method for valproic acid. Dissolve 0.100 g in 25 mL of alcohol. Add 2 mL of water. Titrate with 0.1 M sodium hydroxide determining the endpoint potentiometrically. Each 1 mL of 0.1 M sodium hydroxide is equivalent to 14.42 mg of C8H16O2.

4.4.2. Dosage forms

4.4.2.1. Capsules

The USP [12] recommends the following GC method.

Internal standard solution. Dissolve a quantity of biphenyl in n-heptane to obtain a solution having a concentration of about 5 mg/mL.
Standard solution. Dissolve an accurately weighed quantity of United States Pharmacopoeia valproic acid reference solution in \(n\)-heptane to obtain a solution having a known concentration of about 2.5 mg/mL. Transfer 5.0 mL to a container equipped with a closure. Add 2.0 mL of internal standard solution, close the container, and mix.

Assay preparation. Transfer not less than 20 Capsules to a blender jar or other container, and add about 150 mL of methylene chloride, and cool in a solid carbon dioxide-acetone mixture until the contents have solidified. If necessary, transfer the mixture of capsules and methylene chloride to a blender jar, and blend with high-speed blender until all the solids are reduced to fine particles. Transfer the mixture to a 500-mL volumetric flask, add \(n\)-heptane to volume, mix, and allow solids to settle. Transfer an accurately measured volume of this solution, equivalent to 250 mg of valproic acid, to a 100 mL volumetric flask, dilute with \(n\)-heptane to volume, and mix. Transfer 5.0 mL to a container equipped with a closure. Add 2.0 mL of the internal standard solution, close the container, and mix.

Chromatographic system. The gas chromatograph is equipped with a flameionization detector and a 2 mm \(\times\) 1.8 m glass column packed with 10% phase G34 on 80- to 100-mesh support S1A. The column temperature is maintained at about 150 °C, and the injection port and the detector block temperatures are maintained at about 250 °C. Dry helium is used as the carrier gas at a flow rate of about 40 mL/min. Chromatograph the Standard preparation, measure the peak responses, and calculate the ratio, \(R_S\), as directed for procedure: the relative retention times are about 0.5 for valproic acid and 1.0 for biphenyl; the resolution, \(R\), between valproic acid and biphenyl is not less than 3.0; the relative standard deviation for replicate injections is not more than 2.0%.

Procedure. Separately inject about 2.0 \(\mu\)L each of the standard preparation and the assay preparation, record the chromatograms, and measure the peak responses, for valproic acid and biphenyl peaks. Calculate the quantity, in mg, of C\(_8\)H\(_{16}\)O\(_2\) in the portion of capsules taken using the following formula:

\[
\frac{100C}{R_U} \frac{R_U}{R_S}
\]

in which \(C\) is the concentration, in mg/mL, of United States Pharmacopoeia valproic acid in the standard preparation, and \(R_U\) and \(R_S\) are the peak response ratios obtained from the assay preparation and standard preparation, respectively.

4.4.2.2. Syrup

The USP [12] recommends the following GC method.

Internal standard solution, standard preparation, and chromatographic system are prepared as directed in the assay under valproic acid capsules.

Assay preparation. Transfer an accurately measured volume of syrup, equivalent to about 250 mg of valproic acid, to a separator. Add 40 mL of water and 2.0 mL of hydrochloric acid not less than 20 capsules to a blender jar or other container, and add about 150 mL of methylene chloride, and cool in a solid
carbon dioxide–acetone mixture until the contents have solidified. If necessary, transfer the mixture of capsules and methylene chloride, mix, and extract gently with 80 mL of n-heptane until the aqueous layer is clear (about 3.0 min). Filter n-heptane layer through glass wool, collecting the filtrates in a 100 mL volumetric flask. Rinse the separator and the glass wool with small portions of n-heptane, add the risings to the flask, dilute with n-heptane to volume, and mix. Transfer 5.0 mL to a container equipped with a closure. Add 2.0 mL of the internal standard solution, close the container, and mix.

Procedure. Separately inject about 2.0 μL each of the standard preparation and the assay preparation, record the chromatograms, and measure the peak responses, for valproic acid and biphenyl peaks. Calculate the quantity, in mg, of C₈H₁₆O₂ in each mL of the syrup taken using the following formula:

\[
100C \left(\frac{C}{V}\right) \left(\frac{R_U}{R_S}\right)
\]

in which \(C\) is the concentration, in mg/mL, of USP valproic acid in the standard preparation, \(V\) is the volume, in mL, of syrup taken, and \(R_U\) and \(R_S\) are the peak response ratios obtained from the assay preparation and standard preparation, respectively.

5. REPORTED METHODS OF ANALYSIS

Most of the reported methods of analysis of valproic acid and its sodium salt in biological fluids rely on the use of chromatography, especially gas chromatography, although high performance liquid chromatography (HPLC) is also reported. Other methods, such as flow injection analysis, enzyme-immunoassay, fluorescence-polarization capillary electrophoresis, and potentiometry are sometimes used. The reported methods can be classified as follows.

5.1. Potentiometric method

Sodium valproate has been determined in pharmaceuticals using a valproate selective electrode [13,14]. The electroactive material was a valproate–methyl–tris (tetradecyl)ammonium ion-pair complex in decanol. Silver–silver chloride electrode was used as the reference electrode. The electrode life span was ≥1 month. Determination of 90–1500 μg/mL in aqueous solution by direct potentiometry gave an average recovery of 100.0% and a response time of 1 min.

5.2. Flow injection analysis

Three anticonvulsant drugs including valproic acid were determined using different dyes as ion-pair reagents. Gentian violet was used for the spectrophotometric detection at 588 nm and acridine orange for the fluorimetric detection at 470 nm after excitation at 297 nm. Calibration graphs were linear for 5–50 μg/mL 2.5 ± 0.50 μg/mL for the spectrophotometric and fluorimetric methods, respectively [15].
Valproic acid was determined in serum by flow injection analysis adopting automated fluoroimmunoassay method for the detection [16]. The serum was mixed with the reagents (β-galactosyl ambelliferone-drug conjugate and β-galactosidase-labeled antiserum). The mixture was heated at 30°C for 16 min and then injected into the carrier reagent. Fluorescence was measured at 450 nm after excitation at 405 nm. The detection limit was 1 µg/L.

5.3. Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance spectroscopy was investigated as a method to screen for organic substances (and metabolites) in patients with indications of a drug overdose [17]. Urine specimens containing valproic acid were examined by 1H-NMR spectroscopy at 300 MHz and the results compared with GC–MS.

5.4. Immunoassay methods

A competitive fluorescence-polarization immunoassay method was described for the monitoring of 12 drugs including valproic acid [18]. Samples (serum or plasma) were deproteinated. Fluorescence from the fluorescein-labeled analyte used as tracer was excited at 488 nm and polarization of light emitted at 531 nm was measured. The calibration was stable for 4 weeks and the coefficient of variation was below 10%. A single measurement took 8–10 min.

The chiral evaluation of five drugs including valproic acid using dry film multilayer technology was performed using immunoassay [19]. The test modules are coated multilayer film chips encased in plastic and contains all the reagents required. After loading into the analyzer, a rotor transports the modules to the pipetting station when plasma or serum samples are automatically pipetted into the test modules, fluorescence measurements are made after 6 min.

A micro enzyme multiplied immunoassay technique was developed for antiepileptics including valproic acid [20]. The assay is performed in a total volume of 75 µL and uses only 3–5 µL of serum. A reagent containing a monoclonal drug antibody, the cofactor NAD and the enzyme substrate was added, followed by the drug and glucose-6-phosphate dehydrogenase. The rate of reduction of NAD to NADH was determined by measuring the absorbance at 340 nm at 3 and 4 min after addition of the conjugate. This rate was proportional to the concentration of the drug for 2–150 µg/mL.

Stamp et al. [21] studied the performance of fluorescence polarization immunoassay reagents for some drugs including valproic acid on a Cobas Fara II Analyzer. The method is comparable to the reference method. The calibration graphs are stable for at least 1 month. 6-Amino–2-propylhexanoic acid was used as a direct single reagent for valproic acid in serum [22]. The fluorescence polarization immunoassays were carried out at room temperature. Serum diluted in phosphate buffer of pH 7.5 containing sodium dodecyl sulphate (SDS). Triton X-100 and NH₃ was mixed with fluorescein-labeled valproic acid in buffer-anti valproic acid antiserum and after 10 min, fluorescence polarization was measured.
Five anticonvulsants including valproic acid were determined by the Abbott TD × fluorescence polarization immunoassay automatic analyzer. Recoveries were 94.8–106% and the coefficients of variations were 1.0–9.7% [23]. Fluorescence polarization immunoassay and enzyme immunoassay were compared for the determination of free valproic acid in serum [24]. Good correlation (R = 0.9992) was obtained between the two assays. Higgins [25] reported on the determination of valproic acid in serum by enzyme immunoassay with use of EMIT reagents and the Abbot ABA-200 analyzer. Responses were rectilinear up to 150 mg/L.

5.5. Capillary electrophoresis

Valproic acid has been determined in human serum using capillary electrophoresis and indirect laser induced fluorescence detection [26]. The extract is injected at 75 mbar for 0.05 min onto a capillary column (74.4 cm × 50 μm i.d., effective length 56.2 cm). The optimized buffer 2.5 mM borate/phosphate of pH 8.4 with 6 μL fluorescein to generate the background signal. Separation was carried out at 30 kV and indirect fluorescence detection was achieved at 488/529 nm. A linear calibration was found in the range 4.5–144 μg/mL (0 = 0.9947) and detection and quantitation limits were 0.9 and 3.0 μg/mL. Polonski et al. [27] described a capillary isotachophoresis method for sodium valproate in blood. The sample was injected into a column of an EKI 02 instrument for separation. The instrument incorporated a conductimetric detector. The mobile phase was 0.01 M histidine containing 0.1% methylhydroxycellulose at pH 5.5. The detection limit was 2 μg/mL.

5.6. Chromatographic methods

5.6.1. Thin-layer chromatography

Valproic acid was determined in plasma by treatment with 2,4-dibromoacetophenone or 2-bromoacetonapthalone and with dicyclohexano-18-crown-6 and heated at 70 °C for 40 min. The solution was subjected to TLC on C8F octyl plates or to high performance TLC on RP 8 254 S or Kieselgel 60 F254 plates with developing solvents of aq. 63% ethanol, aq. 73% ethanol or CHCl3-cyclohexane (2:1), respectively, with detection at 280 or 254 nm for the naphthoylmethyl or phenacyl derivative, respectively. The limits of detection were 9.7 and 4.9 μg/mL of valproic acid for the TLC RP 8 and HPTLC RP 8 plates, respectively. Recovery was 84–92.74% [28].

5.6.2. Ion chromatography

Valproic acid was determined in tablets and plasma using ion-chromatography [29]. The extract was injected onto a column (6.5 cm × 6 mm) of Dionex ICE separator resin fitted with a guard column of Aminex cation exchange resin and operated with aq. 0.5 mM CO2 as mobile phase (0.7 mL/min) and conductivity detection. For tablets, the calibration graph was rectilinear for 0.2–25 μg/mL with limit of detection of 50 μg/mL. For plasma, the response was linear for 50–200 μg/mL and limit of detection was 2 μg/mL.
5.6.3. Micellar electrokinetic capillary chromatography

Lee et al. [30] described a micellar electrokinetic capillary chromatographic method for the determination of some antiepileptics including valproic acid. They used a fused silica capillary column (72 cm × 50 μm) and SDS as the micellar phase and multiwavelength UV detection. Reaction conditions, such as pH and concentration of running buffer were optimized. Solutes were identified by characterizing the sample peak in terms of retention time and absorption spectra. Recoveries were 93–105%.

5.6.4. High performance liquid chromatography

Several HPLC methods have been described for the determination of valproic acid, either per se, in formulation or in biological fluids. These methods have been abridged in Table 6.

5.6.5. Gas chromatography

Valproic acid, like short chain fatty acids is volatile and therefore enormous methods have been recommended for its determination in dosage forms and biological fluids. These methods are shown in Table 7.

6. REPORTED METHODS OF ANALYSIS

6.1. Application and associated history

Valproic acid is the common name for 2-propylpentanoic acid (Epival; usually used as its sodium salt), also referred to as n-dipropylacetic acid, is a simple branched-chain carboxylic acid with unique anticonvulsant properties. Valproic acid was first synthesized in 1882 by Burton [75], but there was no known clinical use until its anticonvulsant activity was fortuitously discovered by Pierre Eymard in 1962 in the laboratory of G. Carraz, which was published by Meunier et al. in 1963 [76].

Valproic acid is an effective agent for control of both absence and primarily generalized tonic-clonic seizures. Valproic acid has recently been licensed for the treatment of diseases other than epilepsy, for example, manic episodes associated with bipolar disorder and migraine. By mouth, valproic acid should be initiated at 600 mg twice daily, preferably after food, increasing by 200 mg/day at 3-day intervals to a maximum. The maximum recommended dose of valproic acid is 60 mg/kg/day [77]. By intravenous injection or infusion same as oral route but the injection should be given over 3–5 min. In addition to acid form, the drug is available in several forms, including the sodium or magnesium salts and a combination of acidic form and its sodium salt (divalproex sodium) or an amide form (valpromide). For clinical used the drug is available in capsule, tablet, enteric-coated tablet, sprinkle-capsule, syrup, intravenous, suppository, and sustained-release formulations.

Several mechanisms have been suggested for the action of valproic acid, including blockade of voltage-dependent Na⁺ channel, potentiation of γ-aminobutyric acid
Table 6. Reported HPLC method for valproic acid and sodium valproate

<table>
<thead>
<tr>
<th>No.</th>
<th>Material</th>
<th>Column</th>
<th>Mobile phase</th>
<th>Flow rate</th>
<th>Detection</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rat liver</td>
<td>Micro Bondapka C18 ODS</td>
<td>16.9 mM sodium pyrophosphate (pH 6.9) containing 10–45% acetonitrile</td>
<td>3 mL/min</td>
<td>258 nm</td>
<td>[31]</td>
</tr>
<tr>
<td>2</td>
<td>Serum</td>
<td>YMC-Pak ODS-A (25 cm × 4.6 mm) 5 μm</td>
<td>Methanol–20 mM sodium acetate buffer of pH 3.5 (67:33)</td>
<td>1 mL/min</td>
<td>365/440 nm</td>
<td>[32]</td>
</tr>
<tr>
<td>3</td>
<td>Pharmaceutical preparations</td>
<td>3 μm Adsorbsphe (10 cm × 4.6 mm i.d.)</td>
<td>Methanol–acetonitrile–0.1 M sodium acetate buffer of pH 6.5 (1:4:5)</td>
<td>1 mL/min</td>
<td>Coulometric</td>
<td>[33]</td>
</tr>
<tr>
<td>4</td>
<td>Serum</td>
<td>5 μm Zorbax ODS (25 cm × 4.6 mm i.d.)</td>
<td>Aqueous H2SO4 at pH 4.2</td>
<td>0.8 mL/min</td>
<td>210 nm</td>
<td>[34]</td>
</tr>
<tr>
<td>5</td>
<td>Serum</td>
<td>5 μM Spherisorb C18 (15 cm × 4.6 mm i.d.)</td>
<td>Aqueous 73% methanol</td>
<td>0.9 mL/min</td>
<td>265 nm</td>
<td>[35]</td>
</tr>
<tr>
<td>6</td>
<td>Plasma</td>
<td>5 μm Vydac C18 (15 cm × 4.6 mm i.d.)</td>
<td>1 μg/mL Rhodamine 800 solution in methanol–H2O (1:1) of pH 2.4</td>
<td>1 mL/min</td>
<td>674/700 nm</td>
<td>[36]</td>
</tr>
<tr>
<td>7</td>
<td>Plasma</td>
<td>5 μM Zorbax ODS (25 cm × 4.6 mm i.d.)</td>
<td>Acetonitrile–water–0.25 mM imidazole</td>
<td>1.2 mL/min</td>
<td>Chemiluminescence</td>
<td>[37]</td>
</tr>
<tr>
<td>8</td>
<td>Human plasma and serum</td>
<td>Pecosphere 3 × 3 CR C18 (3.3 cm × 4.6 mm i.d.)</td>
<td>Methanol–0.067 M KH2PO4 buffer of pH 5 (3:1)</td>
<td>1.5 mL/min</td>
<td>245 nm</td>
<td>[38]</td>
</tr>
<tr>
<td>9</td>
<td>Serum</td>
<td>5 μm Hypersil ODS (10 cm × 2.1 mm)</td>
<td>Aqueous 80% ethanol</td>
<td>0.3 mL/min</td>
<td>312/695 nm or 322 nm</td>
<td>[39]</td>
</tr>
<tr>
<td>10</td>
<td>Plasma or serum</td>
<td>Pecosphere 3 × 3 C18 3 μm (3 cm × 4.6 mm)</td>
<td>Acetonitrile–0.03 M KH2PO4</td>
<td>Gradient elution</td>
<td>195 nm</td>
<td>[40]</td>
</tr>
<tr>
<td>11</td>
<td>Capsules</td>
<td>Hypersil RP-8, 5 μm (15 cm × 4.6 mm)</td>
<td>Aqueous 68% acetonitrile</td>
<td>1 mL/min</td>
<td>300/460 nm</td>
<td>[41]</td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>12</td>
<td>Serum</td>
<td>Ultrosphere TM ODS (5 μm)</td>
<td>Aqueous 80% methanol</td>
<td>1.5 mL/min</td>
<td>254 nm</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Mouse brain</td>
<td>Superphase RP-18 (12.5 cm × 6 mm) 4 μm</td>
<td>Methanol–acetonitrile–water</td>
<td>1 mL/min</td>
<td>370/455 nm</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Raw material</td>
<td>RP-8 (5 μm) 10 cm × 4.6 mm i.d.</td>
<td>Methanol–phosphate buffer pH 7 (3:7)</td>
<td>1 mL/min</td>
<td>355/460 nm</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Human plasma</td>
<td>Microporasil 10 μm (30 cm × 3.9 mm)</td>
<td>Hexane–chloroform (47:3)</td>
<td>1.3 mL/min</td>
<td>254 nm</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Blood, serum and brain</td>
<td>Chromospher ODS (20 cm × 3 mm) 5 μm</td>
<td>Acetonitrile–2.5 M formic acid (3:1)</td>
<td>0.4 mL/min</td>
<td>325/398 nm</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Plasma</td>
<td>Lichroprep RP-8 5 to 20 μm</td>
<td>Methanol–water (4:1)</td>
<td>Gradient elution</td>
<td>330/395 nm</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Serum</td>
<td>Shimpack CLC-ODS, 5 μm (15 cm × 6 mm)</td>
<td>Methanol–acetonitrile–H₂O (23:11:6)</td>
<td>1 mL/min</td>
<td>303/376 nm</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Human plasma</td>
<td>Zorbax ODS (pH 5 μm (15 cm × 4.6 mm)</td>
<td>0.01 M NaH₂PO₄ (pH 1.3)–acetonitrile (63:37)</td>
<td>2.5 mL/min</td>
<td>210 nm</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Plasma</td>
<td>LiChrosorb RP-18 (25 cm × 4 mm) 5 μm</td>
<td>Methanol–tetrahydrofuran–0.05 M phosphate buffer pH 5.9 (44:1:55)</td>
<td>1.1 mL/min</td>
<td>201 nm</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Serum</td>
<td>Sil C₁₈, 5 μm</td>
<td>Aqueous acetonitrile</td>
<td>1 mL/min</td>
<td>245 nm</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Dosage forms</td>
<td>Microsorb-MV C₁₈ (25 cm × 4.6 mm i.d.) 5 μm</td>
<td>Acetonitrile–methanol–water (5:2:3)</td>
<td>2 mL/min</td>
<td>245 nm</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Human plasma</td>
<td>RPC₁₈ (25 cm × 4.6 mm) 5 μm</td>
<td>Acetonitrile–0.05 M phosphate buffer pH 3 (45:55)</td>
<td>4.2 mL/min</td>
<td>380 nm</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Human plasma</td>
<td>RPC₁₈ (25 cm × 4.6 mm) 5 μm</td>
<td>Acetonitrile–phosphate buffer pH 3 (45:55)</td>
<td>1.2 mL/min</td>
<td>380 nm</td>
<td></td>
</tr>
</tbody>
</table>
### Table 7. Reported gas chromatographic methods for valproic acid

<table>
<thead>
<tr>
<th>No.</th>
<th>Material</th>
<th>Column</th>
<th>Carrier gas</th>
<th>Detection</th>
<th>Parameter</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tears or plasma</td>
<td>DB5-MS capillary (30 m x 0.25 mm i.d.; 0.25 µm film)</td>
<td>H₂ (1.5 mL/min)</td>
<td>Electron capture negative CIMS</td>
<td>1–15 µg/mL and 1–20 µg/L in plasma and tears, LOD 20 µg/mL</td>
<td>[55]</td>
</tr>
<tr>
<td>2</td>
<td>Human plasma</td>
<td>Chromosorb W HP column packed with 1.5% OV 210 (2 m x 2 mm i.d.)</td>
<td>N₂ (43 mL/min)</td>
<td>FID</td>
<td>10–150 µg/mL, LOD 0.5 µg/mL</td>
<td>[56]</td>
</tr>
<tr>
<td>3</td>
<td>Blood, bile</td>
<td>0.5 µm column (15 m x 0.33 mm i.d.) coated with polyethylene glycol operated at 14°C</td>
<td>H₂ (20 mL/min)</td>
<td>FID</td>
<td>10–150 µg/mL, LOD 0.5 µg/mL</td>
<td>[57]</td>
</tr>
<tr>
<td>4</td>
<td>Serum</td>
<td>0.5 µm Column (15 m x 0.53 mm i.d.) nitrophthalic acid bonded phase capillary</td>
<td>He, H₂, and air (20, 20, and 300 mL/min)</td>
<td>FID</td>
<td>Recovery 84–101%</td>
<td>[58]</td>
</tr>
<tr>
<td>5</td>
<td>Rat plasma</td>
<td>0.25 µm Column (15 m x 0.25 mm i.d.) coated with stabilwax-DA</td>
<td>H₂</td>
<td>FID</td>
<td>Range 0.1–1.0 µg/mL, LOD 0.1 µg/mL</td>
<td>[59]</td>
</tr>
<tr>
<td>6</td>
<td>Plasma of brain homogenate</td>
<td>0.35 µm column (30 m x 0.32 mm i.d.) coated with stabilwax</td>
<td>H₂ (45 cm/s)</td>
<td>ECD</td>
<td>1 pg to 100 ng</td>
<td>[60]</td>
</tr>
<tr>
<td>7</td>
<td>Brain tissue and serum</td>
<td>Fused silica column (30 x 0.75 mm i.d.) coated with DB 1701 (0.25 µm)</td>
<td>H₂ (1 mL/min)</td>
<td>FID</td>
<td>LOD 0.5 µg/mL</td>
<td>[61]</td>
</tr>
<tr>
<td>8</td>
<td>Plasma</td>
<td>Carbowax column (15 x 0.53 mm i.d.) 1 µm operated at 190°C</td>
<td>N₂ (8 µL/min)</td>
<td>FID</td>
<td>1–200 µg/mL, RSD 1.32–1.5%</td>
<td>[62]</td>
</tr>
<tr>
<td>9</td>
<td>Human plasma</td>
<td>GC column (30 m x 0.22 mm i.d.) coated with Nuco (0.25 µm)</td>
<td>Not given</td>
<td>FID</td>
<td>2–20µg/mL, limit of detection 1µg/mL, RSD 1.35%</td>
<td>[63]</td>
</tr>
<tr>
<td>10</td>
<td>Serum</td>
<td>Fused-silica capillary (25 m) coated with SB-11</td>
<td>H₂ (4 mL/min)</td>
<td>CIMS</td>
<td>25–5000 µg/mL, LOD 10 ng/mL, RSD 1.1–1.3%</td>
<td>[64]</td>
</tr>
<tr>
<td>No.</td>
<td>Sample Type</td>
<td>Column Description</td>
<td>Carrier Gas</td>
<td>Detection Method</td>
<td>LOD</td>
<td>CoV</td>
</tr>
<tr>
<td>-----</td>
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<td>--------------------</td>
<td>-------------</td>
<td>-----------------</td>
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</tr>
<tr>
<td>11</td>
<td>Serum and urine of sheep</td>
<td>GC column (30 m × 0.25 mm i.d.) coated with DB-1701 (0.25 μm)</td>
<td></td>
<td>MS</td>
<td>0.01–4 μg/mL, LOD 3 ng/mL, RSD &lt;10%</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Serum</td>
<td>GC column (25 m × 0.2 mm i.d.) coated with HPI (0.33 μm)</td>
<td>H₂ (7.5 kPa)</td>
<td>SIM</td>
<td>LOD was in the low ng/mL, metabolite 100 ng/mL</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Tablet</td>
<td>U-tube glass column (2 m × 3 mm i.d.) of Chromosorb AW-DMCS (80-100 mesh coated with 10% PEG-20 M plus 2% H₃PO₄)</td>
<td>N₂ (50 mL/min)</td>
<td>FID</td>
<td>Range 2–4 μg/mL</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Serum</td>
<td>GC column (25 m × 0.31 mm) coated with HP-5 (0.25 μm)</td>
<td>H₂</td>
<td>FID</td>
<td>LOD 51 pg, recovery &gt;84%</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Plasma</td>
<td>GC column (1.2 m × 4 mm)</td>
<td>N₂ (0.55 kg/cm²)</td>
<td>FID</td>
<td>Range 5–150 mg/L, LOD 2 mg/L</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Serum and urine</td>
<td>GC column (30 m × 0.53 mm) coated with DB 1701 (1 μm)</td>
<td>H₂ (20 mL/min)</td>
<td>EIMS</td>
<td>Range 1–120 μg/mL, coefficient of variation 8%, recovery 90%</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Serum</td>
<td>GC column (30 m × 0.25 mm) coated with SPB 20</td>
<td>H₂</td>
<td>MS</td>
<td>Range 3.5–35 μg/mL</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Serum</td>
<td>Fused silica column (25 m × 0.2 mm) coated with 5% phenylmethylsilicone germ phase HP-5 (0.33 μm)</td>
<td>N₂</td>
<td>FID</td>
<td>Range 0.01–0.1 μg/mL</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Urine, saliva, serum</td>
<td>Bonded-phase column (25 m × 0.32 mm) of OV-1701 (0.25 μm)</td>
<td>H₂</td>
<td>Negative ion Cl–MS</td>
<td>The method determined and identified valproic acid and 15 of its metabolites</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Serum</td>
<td>Steel column (1 m × 4 mm) of 3% of OV 17 on Gas-Chrom Q (80–100 mesh)</td>
<td>N₂ (50 mL/min)</td>
<td>FID</td>
<td>Range 2–12 μg/mL, recovery 97%, coefficient of variation 0.5%</td>
<td></td>
</tr>
</tbody>
</table>
(GABA) mechanisms, and blockade of glutamatergic mechanisms. Because epilepsy is a disease with multiple etiologies, these combinations of mechanisms explain the clinical efficacy of this drug in this disease [78].

6.2. Absorption and bioavailability

Valproic acid is rapidly absorbed after oral administration is provided using conventional formulations. It is found in serum, brain, cerebrospinal fluid (CSF), urine, saliva, breast milk, placenta, and fetal tissue in considerable levels. While on the contrary, it is almost completely bioavailable in human plasma. Hence valproic acid has a high degree of ionization at pH 7.4, it is much less lipid soluble than other anticonvulsant [79]. Twenty percent of the plasma drug is concentrated in the brain and CSF [80]. Placental transfer studies indicate that parent compound and some metabolites are present in cord blood in higher concentrations than in maternal blood [81]. The levels of the drug in fetal circulation and placental tissues were found to be 28 ± 4 and 7 ± 3% [82, 83]. Usually the levels of drugs in cord blood are equal or lower than that of maternal blood. Drug concentration in the breast milk was found to be only 3% of maternal plasma concentration [84]. Valproic acid is strongly bound to serum albumin (>92%) [85].

6.3. Metabolism

Valproic acid is predominantly cleared by biotransformation to give over 50 known metabolites that exert anticonvulsant activity [80]. Although valproic acid is a simple fatty acid, its metabolism is complex with a variety of overlapping phases I and II pathways. It undergoes metabolism by a variety of oxidation and conjugation processes, which result in formation of unsaturated and oxygenated fatty acid metabolites. Of the metabolites of valproic acid, the unsaturated compounds 4-en-valproic acid and trans-isomer of 2-en-valproic acid were 60–100% as potent as the parent drug. Other metabolites are less lipid-soluble than the parent compound; therefore, the brain concentrations of the metabolite are too low to produce any significant anticonvulsant activity [86]. However, they involve in the side effect and toxicity of valproic acid including neurotoxic and hepatic side effects [87,88]. In human, valproic acid is metabolized mainly by cytochrome P450 isoenzymes [89].

The following scheme summarizes the metabolic profile of valproic acid [84].

6.4. Excretion

The main route of excretion of the drug and its metabolites is the kidney with a half-life of 9–18 h in human. In contrast to human, animal models have a lower elimination half-life ranging from 0.6–9 h [78]. The elimination half-life of valproic acid and some metabolites was found to be much longer in the neonates (40–50 h) than adult subjects (9–18 h) [78,81]. One study reported no difference between the elimination half-life between elderly and young subjects (15.4 and 13.0 h, respectively) while other found an increase in for older patients (14.9 versus 7.2 h for young patients) [78,90]. Insignificant amounts of valproic acid are found in breast milk, approximately 3% of maternal drug levels [84].
Valproic Acid and Sodium Valproate: Comprehensive Profile
6.5. Distribution and protein binding

The drug is highly bound to albumin (approximately 90%) [91]. Protein binding is concentration dependent and decreases at high valproate concentration [91]. Free fraction plasma protein concentration increases from approximately 10% at 40 μg/mL to 18.5% at 130 μg/mL [91]. Protein binding decreased markedly in elderly [92], in patients with renal failure [92], and in liver diseases [93].

REFERENCES

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