Analysis of Nanogram Levels of Barbiturates

Analyse von Barbituraten im Nanogramm-Bereich

Analyse de barbiturates au niveau des nanogrammes

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Summary: Nanogram levels of barbiturates can readily be analysed by the addition of formic acid vapour to the carrier gas.

This sample treatment eliminates the problem of adsorption of these strongly polar compounds by active sites and allows symmetrical peaks to be obtained.

By this method it was found that the retention times of the barbiturates were independent of sample size. It is shown that the addition of acid vapour to the carrier gas has no adverse effect on the performance of an Apiezon L column when used with a mixture of six barbiturates.


Dieses einfache Verfahren vermeidet das Problem der Adsorption dieser stark polaren Verbindungen an aktiven Zonen, und die so erhaltenen Peaks sind symmetrisch.

Durch diese Methode ließ sich ermitteln, daß die Retentionszeiten der Barbiturate vom Probenvolumen unabhängig waren. Es wird gezeigt, daß die Zusammensetzung von Säuredampf zum Trägergas bei der Analyse einer Mischung aus sechs Barbituraten keine nachteilige Wirkung auf die Leistung der Apiezon-L-Säule hat.

Sommaire: Les niveaux nanogramme de barbiturate peuvent facilement être analysés par l'addition de la vapeur de l'acide formique au gaz vecteur.

Ce traitement simple élimine le problème d'adsorption de ces matières fortement polaires aux endroits actifs exposés et permet des pics symétriques d'être obtenus.

Par cette méthode on a trouvé que les temps de rétention des barbiturates sont indépendants de la taille d'échantillon. On a montré que l'addition de l'acide n'a point d'effet adverse sur l'efficacité de séparation d'une colonne Apiezon L lorsqu'elle s'utilise avec une mélange de six barbiturates.

One of the problems encountered in gas chromatography is the analysis of strongly polar materials such as barbiturates. Most of the problems associated with the analysis of these compounds are caused by physical adsorption onto active sites in the column system and since the major part of the surface area which comes into contact with the sample is the “inert” support material, particular attention has been paid to this part of the system.

Brookmann-Hansen & Svendsen [1] examined several different liquid phases and reported a separation of 23 barbiturates using two columns of Apiezon L and neopentyl glycol sebacate. Leach and Toseland [2] again used Apiezon L for the barbiturate separation and stressed the importance of acid washing of the support material and removal of “fines”. Anders [3] using a DC200 column stated that column priming with large amounts of barbiturate was necessary before low levels could be analysed.

Cieplinski [4] and McMartin and Street [5, 6] improved recovery of barbiturate from the column by the addition to the liquid phase of dimer acid in the first instance and tristearin in the latter.

Braddock and Marec [7] reported peak tailing at low levels of barbiturate and an increase in retention time below 50 ng. Gudzinowicz and Clarke [8] also showed that below the 0.01 µg level the barbiturate peaks are asymmetric and that the retention time of a given barbiturate increases with decreasing amount of substance injected on to the column.

Blackmore [9] who was interested in determining barbiturates at the nanogram level for forensic purposes used columns of neopentyl glycol adipate with additions of trimer acid (a Cs₄ tribasic acid) and demonstrated the detection of barbiturate in urine at 1 mg/l levels by direct injection into the gas chromatograph.

Pre-silanisation of the glass column and glass wool plugs and column priming with barbiturate were recommended before low level determinations were carried out.

The reported work on barbiturates indicates that there was marked adsorption of these compounds in the column thus making quantitative analysis difficult and trace analyses at levels below 50 ng frequently impossible because of complete loss on the system.

Because of the similarity in behaviour of barbiturates and free fatty acids in showing loss by adsorption, the technique of adding formic acid vapour to the carrier gas, after Ackman & Burgher [10] was investigated.

They approached the problem of fatty acid analysis by adding formic acid vapour to the carrier gas from a cold-finger trap placed in the gas stream immediately before the injector. The advantage of this method of de-activation is that, apart from obvious cases of interaction, the liquid phase giving the best separation can be chosen whether it be polar or non-polar.
Experimental

All chromatograms were obtained on a Perkin-Elmer Model F11 fitted with an Analyzer No. 7 suitable for 1/4 in glass columns with on-column injection and flame ionization detector.

Since Apiezon L has been shown to be fairly selective for barbiturates a 6 ft x 1/4 in glass column was packed with 4% Apiezon L on 80–100 AW.DMCS. Chromosorb W and conditioned before use at 230 °C for 24 hours.

Other operating conditions were as follows:

- Column Temp: 185 °C
- Injector Temp: 260 °C
- Carrier Gas: Nitrogen at X 20 lbf/in² inlet pressure
- Detector Temp: 185 °C
- Hydrogen: 18 lbf/in²
- Air: 25 lbf/in²
- Sample: Barbiturate in Chloroform solvent

A solution containing 189 ng/µl of hexobarbitone and 185 ng/µl of octadecane was prepared and was used to test the system. Hexobarbitone was chosen since Gudzinowicz and Clarke has shown this to exhibit, in common with other barbiturates, the dependence of retention times on amount of sample added. Octadecane was included as a convenient marker.

Fig. 1 shows a chromatogram of 1 µl of the test solution using carrier gas without the addition of formic acid. Only the hydrocarbon peak was obtained and the effect was repeated for several injections. Thus it can be seen that the system was removing 189 ng of hexobarbitone per injection.

Fig. 2 shows 1 µl of the same solution after the addition of formic acid vapour to the carrier gas. This was conveniently done by allowing the gas to flow over 5 ml of formic acid contained in a 25 ml glass trap which was connected directly into the chromatograph via the normal inlet for the carrier gas.

Under these conditions the first injection produced a symmetrical peak for hexobarbitone, with a retention time of 5.3 min indicating that the acid vapour was effective in blocking the active sites causing loss of the barbiturate.

Since the addition of formic acid vapour proved effective in de-activating the column, as shown by the levels of barbiturate estimated, it was decided to investigate the degree of de-activation compared with the amount of formic acid vapour in the carrier gas.

Table 1
Effect of formic acid concentration on retention time

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Retention Times min</th>
<th>Relative Ret. Time</th>
<th>Peak Height cm</th>
<th>Ht. Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C18</td>
<td>Hexobarb.</td>
<td>C18</td>
<td>Hexobarb.</td>
</tr>
<tr>
<td>18 °C</td>
<td>3.5</td>
<td>5.3</td>
<td>1.52</td>
<td>9.2</td>
</tr>
<tr>
<td>25 °C</td>
<td>3.6</td>
<td>5.35</td>
<td>1.49</td>
<td>8.85</td>
</tr>
<tr>
<td>40 °C</td>
<td>3.6</td>
<td>5.4</td>
<td>1.50</td>
<td>8.95</td>
</tr>
<tr>
<td>55 °C</td>
<td>3.6</td>
<td>5.4</td>
<td>1.50</td>
<td>8.8</td>
</tr>
</tbody>
</table>

Fig. 1
- Test solution analysed using pure nitrogen carrier gas
- Analyse einer Testlösung mit reinem Stickstoff als Träergas
- Analyse d'une solution d'essai, avec de l'azote pur comme gaz vecteur