Evaluation of selected analyte protectant to improve performance of gas chromatographic analysis of pesticide residues
(Penilaian pelindung anilit terpilih untuk meningkatkan prestasi analisis kromatografi gas residu pestisid)

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Abstract
The effectiveness of three analyte protectants, namely, D-sorbitol, 3-ethoxy-1,2-propanediol (ethoxypropanediol) and L-gulonic acid γ-lactone (gulonolactone) in improving peak shape of analytes for better peak sensitivity and quantitation during GC-MS analysis of pesticide residues were evaluated. The use of these analyte protectants were investigated with three pesticide groups i.e. organochlorine (γ-HCH and α-endosulphan), organophosphate (chlorpyriphos-methyl, fenitrothion and prothiophos) and synthetic pyrethroid (bifenthrin, fenpropathrin, cypermethrin and deltamethrin). The analyte protectants were fortified in mixed pesticide standard at a rate of 1 mg/ml prior to GC-MS analysis. Improvement of analytes was seen as peak was enhanced as much as 41% and better separation obtained with application of analyte protectants. These compounds fill in the active sites in the inlet and column of the GC-MS, thus reducing analyte interactions. The ethoxypropanediol seems adequate to provide protection for pesticide during early to mid-eluting process while sorbitol and gulonolactone can be used for mid to late-eluting process. However, a combination of all three analyte protectants will provide better protection.

Introduction
One of the major problems in pesticide residue analysis using gas chromatography (GC) is analyte loses (Mastovska et al. 2004; Dömötörováa and Matisova 2008; Przybyliski and Hommet 2008). This problem arises due to interaction of analytes with active sites in the GC inlet and column during elution process resulting in poor peak shape which is difficult to identify and quantify because of susceptibility to interference (Hajslova and Zrostlikova 2003; Hoh and Mastovska 2008). Recent studies by Anastassiades et al. (2003) showed that by applying analyte protectant, peak distortion in fruits and vegetables matrices was significantly enhanced. These analyte protectants help to improve peak shape by filling in the active site thus reducing analyte interactions (Rodriguez et al. 2008).

This study explored the possibility of three analyte protectants: Gulonolactone, D-sorbitol and 3-ethoxy-1,2-propanediol in improving peak shape of various pesticide residues by gas chromatographic analysis. Three groups of pesticide were evaluated which consist of organochlorine group (γ-HCH and α-endosulphan), organophosphorous group (chlorpyriphos, fenitrothion and prothiophos) and synthetic...
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pyrethroid group (bifenthrin, cypermethrin, deltamethrin and fenpropathrin).

Materials and methods
Chemicals bought from Merck were anhydrous sodium sulphate and D-sorbitol, both with at least 95% purity. Chemicals bought from Sigma Aldrich were 3-ethoxy-1,2-propanediol with 98% purity and L-gulonic acid γ-lactone with 89.5% purity. Others were primary secondary amine (PSA) powder with 99.9% purity from Bondensil, sodium hydrogen carbonate with 97% purity from Ajax Chemicals, anhydrous magnesium sulphate with 97% purity from Acros Organics. Pesticide grade solvent used in this study, ethyl acetate n-hexane, was purchased from Merck.

All glassware and apparatus used in the analysis were soaked in Decon for at least 12 h (Anastassiades et al. 2003). Then they were rinsed with tap water followed by deionised water and acetone and dried in the oven for at least 2 h at 60 °C. Later they were wrapped with aluminium foil to avoid contamination.

Pesticide standards used in this study were purchased from Riedel-de Haen including α-endosulphan (99.8% purity), bifenthrin (99.9% purity), cypermethrin (95.6% purity), chlorpyriphos (99.6% purity), deltamethrin (99.8% purity), fenitrothion (96.3% purity) and fenpropathrin (98.5% purity). Standards purchased from Dr. Ehrenstorfer include γ-HCH (99.4% purity) and prothiophos (94% purity).

Pesticide standard stock solutions were prepared by first adjusting to 100% purity to achieve 1,000 mg/litre (1,000 ppm) and dissolved in ethyl acetate. This standard stock solution was then kept in the freezer at temperatures between –16 °C and 4 °C.

The working standards were prepared by diluting the standard stock solution into a 10 ml volumetric flask using hexane as a solvent. Concentrations of working standards in this study were between 0.2 and 1 mg/litre. These standards provide qualitative information on retention time during gas chromatographic analysis.

The mixed pesticide standards were prepared by transferring the stock standard into 50 ml volumetric flasks and topped up using acetone as the solvent. These mixed standards were used to spike samples in recovery studies and as external standards for quantitative works.

Each of the analyte protectant (APs) was first adjusted to 100% purity to achieve 400 mg/ml (400 ppt) and dissolved in deionised water. This APs stock solution was then kept in the refrigerator at temperatures between –2 °C and 4 °C.

Each of the APs stock solution was transferred into 10 ml volumetric flask and topped up with acetone. Acetone was used instead of acetonitrile due to immiscibility between acetonitrile and ethyl acetate. Since most of the solvents used in this study were hexane and ethyl acetate, acetone was preferred since it was miscible in hexane and ethyl acetate.

Analyte protectant was added in between of clean-up and pre-concentrate step at a concentration of 1 mg/ml using the micropipette.

The GC-MS used in the study, Perkin Elmer 600 series, was set with the following parameters:

Detector: Mass spectrometer (Quadrupole)
Column: DB-5MS (cross-linked 5% Phenyl Methyl Silicone)
Column phase ratio: 250
Column film thickness: 0.25 µm
Column length: 30 m
Column internal diameter: 0.25 mm
Carrier flow (Helium): 1 ml/min
Injection volume: 2 µl
Injector temperature: 280 °C
Inlet transfer line temperature: 300 °C
Ion source temperature: 300 °C

The temperature programme was set with an initial temperature of 50 °C which was...
held for 1 min. Then it was increased to 190 °C at a rate of 50 °C/min and held for 2 min. Later, the temperature was increased to 320 °C at a rate of 5 °C/min and held for 5 min.

For tuning purposes, heptacosa was used as a reference due to its capability to cover 45 – 650 amu. Tuning was done automatically using GC-MS software, Turbomass.

The GC-MS injection sequence was performed initially with acetone as blank. Then injection of standards was done first with individual pesticide standards, followed by a series of mixed standards and mixed standards mixed with APs (Anastassiades et al. 2003). Blank injections were performed between all the set of injections above in order to identify possible carry over.

A series of standards in the range of 0.2 ppm until 1.0 ppm was prepared and injected into GC-MS to get linearity of the signal generated. From the series of injected standards, a calibration curve was obtained using \( y = mx + c \) equation. After this, linear regression \( (R^2) \) value was obtained using this equation, \( R^2 = 1 - \frac{SS_{err}}{SS_{tot}} \) where \( SS_{err} \) is the residual sum of squares and \( SS_{tot} \) is the total sum of squares (Gonzalez and Herrador 2007). For this study, minimum acceptable \( R^2 \) value was set at 0.95. If the value was less than 0.95, the standards will be re-prepared and re-injected.

Results and discussion

Calibration curve of standards

Figure 1 shows an example of a calibration curve of standards which was developed for the various pesticides. \( R^2 \) for organochlorine pesticides, \( \gamma \)-HCH and \( \alpha \)-endosulphan, were 0.9972 and 0.9975 respectively (Table 1). On the other hand, organophosphate group pesticides which consists of chlorpyriphos-methyl, fenitrothion and prothiophos gives linearity values of 0.9977, 0.9908 and 0.9981 respectively. This excellent linearity can also be seen for the synthetic pyrethroid group which consists of bifenthrin, fenpropathrin, cypermethrin and deltamethrin with values of 0.9997, 0.9987, 0.9992 and 0.9985 respectively.

Comparison of \( R^2 \) of standards fortified with analyte protectant

In this study, three potential analyte protectants were evaluated: D-sorbitol, L-gulonic acid \( \gamma \)-lactone and 3-ethoxy-1,2-propanediol. Each compound was evaluated in terms of its ability to improve linearity of GC-MS signal, \( R^2 \). Fortifications of all APs were done at a concentration of 1 mg/ml.

To compare the \( R^2 \) of standards, 2 \( \mu l \) of mixed pesticides standards were injected into GC-MS together with mixed pesticides standards added with APs individually. \( \gamma \)-HCH improved greatly with addition of ethoxypropanediol with an \( R^2 \) value of 0.999 followed by sorbitol with a value of 0.994 (Table 2 and Figure 2). However, gulonolactone gives negative effect with a reduction of \( R^2 \) from 0.992 in standards

![Figure 1. Calibration curve of standard for cypermethrin](image)

Table 1. \( R^2 \) of standards

<table>
<thead>
<tr>
<th>Compound</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \gamma )-HCH</td>
<td>0.9972</td>
</tr>
<tr>
<td>Chlorpyriphos-methyl</td>
<td>0.9977</td>
</tr>
<tr>
<td>Fenitrothion</td>
<td>0.9908</td>
</tr>
<tr>
<td>( \alpha )-endosulphan</td>
<td>0.9975</td>
</tr>
<tr>
<td>Prothiophos</td>
<td>0.9981</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>0.9997</td>
</tr>
<tr>
<td>Fenpropathrin</td>
<td>0.9987</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>0.9992</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>0.9985</td>
</tr>
</tbody>
</table>
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Table 2. Comparison of R² of standards fortified with analyte protectant

<table>
<thead>
<tr>
<th>Standards</th>
<th>Standard without APs</th>
<th>Standard with sorbitol</th>
<th>Standard with ethoxypropanediol</th>
<th>Standard with gulonolactone</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ-HCH</td>
<td>0.992</td>
<td>0.994</td>
<td>0.999</td>
<td>0.228</td>
</tr>
<tr>
<td>Chlorpyriphos-methyl</td>
<td>0.994</td>
<td>0.172</td>
<td>0.999</td>
<td>0.996</td>
</tr>
<tr>
<td>Fenitrothion</td>
<td>0.991</td>
<td>0.149</td>
<td>0.995</td>
<td>0.992</td>
</tr>
<tr>
<td>α-endosulphan</td>
<td>0.991</td>
<td>0.575</td>
<td>0.998</td>
<td>0.994</td>
</tr>
<tr>
<td>Prothiophos</td>
<td>0.990</td>
<td>0.946</td>
<td>0.999</td>
<td>0.994</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>0.991</td>
<td>0.894</td>
<td>0.998</td>
<td>0.994</td>
</tr>
<tr>
<td>Fenpropathrin</td>
<td>0.992</td>
<td>0.877</td>
<td>0.997</td>
<td>0.995</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>0.988</td>
<td>0.846</td>
<td>0.994</td>
<td>0.977</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>0.991</td>
<td>0.827</td>
<td>0.936</td>
<td>0.988</td>
</tr>
</tbody>
</table>

Figure 2. Comparison between R² of organochlorine standards with and without addition of analyte protectants

Figure 3. Comparison between R² of organophosphate standards with and without addition of analyte protectants

Figure 4. Comparison between R² of synthetic pyrethroids standards with and without addition of analyte protectants

without APs to a value of 0.228 due to polarity difference (Anastassiades et al. 2003).

On the other hand, chlorpyriphos-methyl gives R² of 0.994 without addition of APs due to its stability and less likely to be retained at injector port (Kirchner et al. 2008). Improvement in R² was seen with addition of ethoxypropanediol (0.999) and gulonolactone (0.996) except sorbitol, which reduced R² to 0.172. This negative effect was due to close polarity between sorbitol-chlorpyriphos-glass wool at injector port, which caused chlorpyriphos to be retained at injector port (Kirchner et al. 2008).

As seen in Figure 3, fenitrothion gave an R² of 0.991 and with addition of ethoxypropanediol and gulonolactone, this value increased to 0.995 and 0.992 respectively. Again, sorbitol gives negative
effect towards fenitrothion with a R² value of 0.149. Nevertheless, the negative effect of sorbitol was reduced as eluting time increased in which R² value of prothiophos, bifenthrin, fenpropadrin, cypermethrin and deltamethrin added with sorbitol were not significantly reduced with values of 0.946, 0.894, 0.877, 0.846 and 0.827 respectively. This is due to the natural stability of these compounds towards heating ramp during GC-MS analysis (Schrecka et al. 2008).

Prothiophos and endosulphan have a R² value of 0.990 and 0.991 respectively (Table 2). Improvement can be seen as R² value of endosulphan increased to 0.992 and 0.998 with addition of gulonolactone and ethoxypropanediol respectively. Linearity of prothiophos is at best with addition of ethoxypropanediol followed by gulonolactone with a value of 0.999 and 0.994 respectively. These two compounds performed well towards prothiophos due to polar effect, which causes prothiophos to be eluted together along with these compounds (Hajslova and Zrostlikova 2003).

The last three compounds that showed positive enhancements as a result of addition of ethoxypropanediol were bifenthrin, fenpropadrin and cypermethrin in which with addition of this agent, its R² values increased from 0.991 to 0.998 for bifenthrin, 0.992 to 0.997 for fenpropadrin and 0.988 to 0.994 for cypermethrin (Table 2). However, gulonolactone only gave enhancements for bifenthrin and fenpropadrin in which it’s R² values increased from 0.991 to 0.994 for bifenthrin and 0.992 to 0.995 for fenpropadrin (Table 2).

Deltamethrin, which is the last eluate pesticide, did not get any sharp enhancement from all the three APs (Figure 4). Only γ-HCH improved with addition of sorbitol. Ethoxypropanediol was observed to enhance most of the pesticides tested other than deltamethrin. Whereas, gulonolactone was effective for all the pesticides tested excluding γ-HCH, cypermethrin and deltamethrin due to polarity differences (Anastassiades et al. 2003). However, to really ascertain enhancement effect of all the APs, a more thorough evaluation has to be made.

**Conclusion**

Three analyte protectants, D-sorbitol, 3-ethoxy-1,2-propanediol (ethoxypropanediol) and L-gulonic acid γ-lactone (gulonolactone) were effective in varying rates in improving peak shape of 3 pesticide groups; organochlorine (γ-HCH and α-endosulphan), organophosphate (chlorpyriphos-methyl, fenitrothion and prothiophos) and synthetic pyrethroids (bifenthrin, fenpropadrin, cypermethrin and deltamethrin) in GC-MS analysis. The R² standard with addition of APs was used to evaluate effectiveness of APs. Sorbitol was able to protect only γ-HCH. On the other hand, ethoxypropanediol was able to enhance peaks of all tested pesticides except deltamethrin. Enhancement by gulonolactone was seen at all tested pesticides excluding γ-HCH, cypermethrin and deltamethrin. This evaluation only indicated a little information about activity and potential of APs. More work has to be done to further evaluate these APs using different approaches as well as long-term influence of using these APs on the GC-MS system.

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**References**


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**Abstrak**

Keberkesanan tiga pelindung anilit, iaitu D-sorbitol, 3-etoksi-1,2-propanediol (etoksi propanediol) dan asid L-gulonik γ-lakton (gulonolakton) dalam pembaikan bentuk puncak anilit bagi meningkatkan sensitiviti puncak dan kuantitasi semasa analisis GC-MS residu pestisid telah dinilai. Penggunaan pelindung anilit telah dikaji menggunakan tiga kumpulan pestisid, iaitu organoklorin (γ-HCH dan α-endosulfan), organofosfat (chlorpirifos-metil, fenitrotion dan protiofos) dan sintetik piretroid (bifentrin, fenpropatrin, cypermetrin dan deltametrin). Pelindung anilit ditambah ke dalam campuran standard pestisid dan diaplikasikan pada kadar 1 mg/ml sebelum dianalisis dengan menggunakan GC-MS. Pembaikan terhadap puncak anilit ditingkatkan sebanyak 41% dan pemisahan puncak yang lebih baik diperoleh menerusi penggunaan pelindung anilit. Sebatian ini bertindak dengan memenuhi tapak aktif di dalam salur masuk dan turus GC-MS, dan seterusnya mengurangkan interaksi anilit. Etoksi propanediol dilihat mampu melindungi pestisid yang terelut awal sehingga pertengahan dan sorbitol serta gulonolakton pula mampu melindungi pestisid yang terelut dari pertengahan hingga lewat. Walau bagaimanapun gabungan ketiga-tiga pelindung anilit ini akan menghasilkan kesan perlindungan yang lebih baik.

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