Different Algorithms for Improving Detection Power of Atomic Fluorescence Spectrometry

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Abstract
The purpose of detecting trace concentrations of analytes often is hindered by occurring noise in the signal curves of analytical methods. This is also a problem when different arsenic species (organic arsenic species such as arsanilic acid, nitarsone and roxarsone) are to be determined in animal meat by HPLC-UV-HG-AFS, which is the basis of this work. In order to improve the detection power, methods of signal treatment may be applied. We show a comparison of convolution with Gaussian distribution curves, Fourier transform, and wavelet transform. It is illustrated how to estimate decisive parameters for these techniques. All methods result in improved limits of detection. Furthermore, applying baselines and evaluating peaks thoroughly is facilitated. However, there are differences. Fourier transform may be applied, but convolution with Gaussian distribution curves shows better results of improvement. The best of the three is wavelet transform, whereby the detection power is improved by factors of about 2.4

Keywords: Limit of detection; Gaussian; Fourier; Wavelet; Atomic Fluorescence Spectrometry

1. Introduction
Arsanilic acid (ASA), nitarsone (4-nitrophenylarsonic acid) (NIT) and roxarsone (4-hydroxy-3-nitrophenylarsonic acid) (ROX) (Fig.1) were the best known arsenic species, which were widely used as feed additives in the poultry industry to control coccidial intestinal parasites [1]. Arsanilic acid and roxarsone were used to prevent and treat coccidiosis in poultry and haemorrhagic enteritis in pigs, nitarsone was the drug of choice to prevent and treat histomoniasis (blackhead), a disease caused by the protozoan Histomonas meleagridis [2]. The use of these organoarsenic additives can cause bioaccumulation potential in human body [3] and it can deduce that repeated annual Poultry litter applications to agricultural soils, especially those used for rice cultivation may result in As buildup in soil, leading to plant uptake and subsequent transfer to the human food chain [4]. Whereas, some studies had disputed the real effect of these arsenic species and showed that there also existed a low bioaccumulation potential in human body [3]. In addition to these findings, numbers of reports had pointed out that some factor can lead these additives to the formation of highly inhibitory compounds [5] which will cause toxic effects when used at higher than recommended levels [6, 7]. What was worse, some studies indicated that roxarsone appears to ultimately decompose to water-soluble toxic arsenicals, primarily as inorganic arsenate, which was virulent to human [8, 9]. To avoid the misuse of this class of species so as to ensure the quality of poultry and livestock industry, arsenic compounds must be monitored.

The high performance liquid chromatography coupled to ultraviolet oxidation hydride generation atomic fluorescence spectrometry (HPLC-UV-HG-AFS) is a substantially required method to estimate different arsenic forms. However, even this technique is limited due to occurring noise in the chromatograms.

When the signal itself cannot be increased, noise should be decreased instead. This can be accomplished by different means of signal treatment. Well known are techniques like moving average for smoothing, but these often contain also the disadvantage of reducing not only noise but also the signal itself. More improved methods are the convolution with Gaussian distribution curves, Fourier transform, and wavelet transform. Former studies on the application of the convolution with Gaussian distribution curves for de-noising have mostly been restricted to artificially noised Gaussian peaks or sine curves [10, 11]. Although Fourier transform (FT) is
used in analytical chemistry rather often, especially for techniques like infrared spectroscopy or Fourier transformed AC voltammetry [12, 13], obviously it is not used for de-noising very often. A further development of FT is wavelet transform [14], used both for de-noising [15–17] and also for peak separation [18–20].

Figure 1. Structures of arsenic species: arsanilic acid, roxarsone, nitarsone.

In this paper, we will show a comparison of all three methods of signal treatment for the capability of de-noising signals. Moreover, this will not be accomplished for artificially noised peaks, but for real chromatograms instead.

2. Experimental

2.1 Instrumentation

A non-dispersive hydride generation-atomic fluorescence spectrometer system of AFS9600 (Beijing Haiguang Instruments Co., Ltd., Beijing, China) was used. The on-line oxidation unit was manufactured in laboratory, constructed of heating filament and six UV lamps enlaced with 5 meters quartz tube. The HPLC system consisted of a Labtech P600 pump (Labtech, USA), a Rheodyne 7725i 6-way valve sample injector (Rheodyne, Cotati, USA) and a Phenomenon C18 column (Luna 5u, 250'4.60 mm).

A Dionex ASE300 accelerated solvent extractor (CA, USA) was applied for sample extraction. pH values were measured with the Mettler-Toledo 320-S pH meter (Mettler-Toledo, Greifensee, Switzerland). An LD5-2A centrifuge (Beijing Medical Centrifuge Factory, Beijing, China) was applied to separating the suspensions.

2.2 Reagents

All reagents used in the whole process were all of analytical-grade and the following chemicals: NaOH (Tianjin Fengchuan chemical Reagent Co., Ltd, China), KBH$_4$ (Laboratory of chemical reagent, Tianjin, China), KH$_2$PO$_4$ (Sinopharm chemical Reagent Co., Ltd, China), Hydrochloric acid (DUKSA NA PURE CHEMICALS, Korea), Methanol (D I K MA TECHNOLOGIES INC., USA), Trifluoroacetic acid (Aifa Aesar, Tianjin, China), Silica sand (Shanghai Tianlian Chemical Reagent Co., Ltd, China). For preparation of organoarsenic species standard solutions Arsanilic acid (Labor Dr. Ehronstorfer, Germany, GmbH, 99.0%), Roxarsone (Labor Dr. Ehronstorfer, GmbH, Germany, 99.0%), Nitarsone (Wako, Japan) were used. Deionized water (Millipore Corporation, USA) was used through all the process.

2.3 Calibration

Calibration for the three arsenic species was performed in the lower working range, i.e., from 0.1 to 2.0 ug mL$^{-1}$, in order to calculate the limits of detection. Ten calibration standards have been measured as well as a blank solution.

3. Theoretical

3.1 Signal-to-noise ratio and limit of detection

Analytical noise is quite often characterized by the standard deviation of blank measurements [21, 22]. For the interpretation, if signals may be seen as detected or not, the SNR can directly be used. On the other hand, it is also possible to calculate the limits of detection. Based on the definition that a measured value has to be greater than the noise to be
regarded as detected, and also considering that half of the noise enters into the computation of the intercept, the LOD can be calculated by Eq. 1.

\[ c_{LOD} = \frac{3 \cdot SD}{a} \]  

(1)

where SD is the standard deviation and a is the slope of the linear calibration.

### 3.2 Convolution with Gaussian distribution curves

Gaussian distribution curves are used as special filters. The reason for their usability is that most analytical peaks are seen as Gaussian distributed in theory. Therefore, cross-correlation, i.e., convolution, may be used for de-noising. Mathematically, cross-correlation coefficients between the original signal curve and Gaussian peaks are calculated (Eq. 2). For this, only a short computing time is necessary.

\[
\Omega_{s g} (\tau) = \lim_{T \to \infty} \frac{1}{2T} \int_{-T}^{+T} s(t) \cdot g(t - \tau) \, dt
\]  

(2)

Figuratively, the Gaussian distribution curve, g(t), is moved along the signal curve, s(t). The higher the correlations between the two, the higher also are the values for Ω. The highest correlation, however, should occur when both peaks are directly at the same position. As there is practically no correlation of Gaussian distribution curves and noise, the signal shape is retained whereas the noise is decreased.

Gaussian distribution curves are defined by only two parameters, the mean value \( \mu \) and the standard deviation \( \sigma \). The latter is to be chosen carefully before de-noising as the Gaussian curve’s half-width, \( w_{0.5} \), is directly proportional to \( \sigma \) (Eq. 3).

\[ w_{0.5} = 2\sqrt{\ln 2 \cdot \sigma} \]  

(3)

Too narrow Gaussian distribution curves do not de-noise well enough as they resemble too much the high frequent noise. Too broad ones, however, will lead to the broadening of signal peaks. According to the literature [23], one should be chosen such that the Gaussian distribution curve’s halfwidth is only 60% of the signal peak’s half-width.

### 3.3 Fourier transform

The principle of Fourier transform is that all periodic functions may be approximated by a sum of the sine and cosine waves. Thereby, the signal is transformed from the time domain into the frequency domain (Eq. 4). In the first step, the original signal s(t) is approximated with a

Table 1 Operating conditions of the HPLC-UV-HG-AFS system

<table>
<thead>
<tr>
<th>Chromatography</th>
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<tr>
<td>HPLC column</td>
<td>Luna 5u C18 (250*4.60 mm)</td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
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<td></td>
</tr>
<tr>
<td>Injection volume</td>
<td>100 uL</td>
<td></td>
</tr>
<tr>
<td>Mobile phase</td>
<td>5.95 v/v Methanol/0.05mol/L KH2PO4 (included 0.1% v/v TFA ) PH:1.67</td>
<td></td>
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<tr>
<td>Mobile phase flow rate</td>
<td>1.2 mL min⁻¹</td>
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<tr>
<th>Hydride generation</th>
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<tr>
<td>Acid solution</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>Reducer</td>
<td>20% KBH4 in 5% KOH</td>
<td></td>
</tr>
<tr>
<td>HCl flow rate</td>
<td>3 mL min⁻¹</td>
<td></td>
</tr>
<tr>
<td>Reducer flow rate</td>
<td>3 mL min⁻¹</td>
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<th>Atom fluorescence spectrometry</th>
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<tbody>
<tr>
<td>Lamp current (mA)</td>
<td>100 mA (primary) 50 mA (assistant)</td>
<td></td>
</tr>
<tr>
<td>Resonance wavelength (nm)</td>
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<td></td>
</tr>
<tr>
<td>Carrier gas</td>
<td>Argon</td>
<td></td>
</tr>
<tr>
<td>PMT voltage (V)</td>
<td>330</td>
<td></td>
</tr>
<tr>
<td>Atomizer height (mm)</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Carrier gas flow (mL min⁻¹)</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td>Shield gas flow (mL min⁻¹)</td>
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<td></td>
</tr>
<tr>
<td>Read mode</td>
<td>Peak area</td>
<td></td>
</tr>
</tbody>
</table>
cosine and a sine wave of the fundamental frequency $\omega$, i.e., with one full period within the analytical signal. As degree of correlation, the coefficients $a_k$ and $b_k$ are calculated, respectively. In the next steps, the sampling frequency is successively increased by multiplying with positive integers $k$, for which particular Fourier coefficients are calculated [24]. Doing so, all present portions of frequency within the signal $s(t)$ are gathered. For completing the Fourier transform, only the constant $a_0$ has to be calculated yet.

$$s(t) = \frac{1}{2}a_0 + \sum_{k=1}^{\infty} (a_k \cos k\omega t + b_k \sin k\omega t)$$  \hspace{1cm} (4)

The Fourier coefficients $a_k$ and $b_k$ are calculated via integrals in general (Eqs. 5 and 6).

$$a_k = 2\int_0^1 s(t) \cos k\omega t \, dt$$  \hspace{1cm} (5)

$$b_k = 2\int_0^1 s(t) \sin k\omega t \, dt$$  \hspace{1cm} (6)

The graphical result of the Fourier transform is the representation of the signal as a frequency spectrum. The original signal may be restored by inverse transform using all coefficients. When the signal is to be de-noised, this should not be done, though. Generally, noise is of higher frequency than analytical peaks. Therefore, omitting coefficients for high frequencies, the signal is de-noised [21]. This is accomplished by multiplying the Fourier transform of the signal with a low-pass filter. The most common one consists of the value 1 up to a certain limit and of the value 0 for the rest of the digits. Thus, the coefficients below the limit are retained; all other ones are eliminated [24]. Afterwards, the inverse Fourier transform results in a de-noised signal. The important point is to find an ideal limit, the cutoff frequency $\nu_0$. If it is too high, too much noise is retained; if it is too low, the signal is deformed [25].

3.4 Wavelet transform

Wavelet transform can be considered as a further development of FT as the latter either allows the interpretation of the frequency domain or of the time domain, although in many cases both types of information are requested. In contrast to FT, where sine and cosine waves reproduce the original signal, WT offers various different functions $\Psi$ to analyze a signal [26]. Wavelets converge to zero relatively fast and they feature the mean value of zero. Most of them are asymmetric and show irregularities, like Daubechies8, which is used in this work. The basic function, called mother wavelet, is modified by two parameters: It may be scaled (dilation, parameter $a$) and shifted (translation, parameter $b$), as can be seen in Eq. 7.

$$\Psi_{a,b} = \frac{1}{\sqrt{a}} \Psi\left(\frac{x-b}{a}\right)$$  \hspace{1cm} (7)

However, in discrete wavelet transform, which is used in this work, the choice of $a$ and $b$ is restricted to

$$a = a_0^l \cdot a_j, \quad b = k \cdot b_0 \cdot a_j$$  \hspace{1cm} (8)

where $k$ and $j$ are positive or negative integers [27]. Quite analogous to FT, wavelet coefficients $c$ are calculated (Eq. 9) with which the original signal $s(t)$ can be restored by inverse transform.

$$c_{a,b} = \int_{-\infty}^{\infty} s(t) \Psi_{a,b}(t) \, dt$$  \hspace{1cm} (9)

Wavelet transform can therefore be described in such a way that the mother wavelet is positioned at every possible location within the signal and the degree of correlation is calculated for each position. In the next steps, the mother wavelet is dilated and again coefficients are calculated for each possible position. In order to analyze the frequency structure of the signal, after the first step, low- and high-pass filters are used. Thus, the signal is split into two parts:
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approximations and details, respectively. This is repeated after subsequent steps. The number of steps, i.e., the level of decomposition, therefore, corresponds to the number of scaling parameters a. This separation can be used when restoring the signal by inverse transform. Again, not all coefficients should be used. As coefficients describing high-frequency components are part of the “details,” those should be omitted in order to achieve de-noised signals. Although filtering is quite similar as described for Fourier transform, typical for wavelet transform is the option of hard or soft thresholding. Using the first one, wavelet coefficients below a certain threshold are cutoff completely, whereas their values are just reduced using soft thresholding [15].

All calculations have been performed using MATLAB (v7.14.0.739, The MathWorks Inc.). For the convolution with Gaussian distribution curves, an adequate function \( g(t) \) has been created using the well-known functional equation of the Gaussian distribution curve and the parameter \( \sigma \) in accordance with to Eq. 3. Then, the function \( g(t) \) is convolved with the signal \( s(t) \) using the command “conv,” resulting in the de-noised signal. De-noising by FT is accomplished by several steps, essentially using the command “fft,” a simple filter, and the command “ifft”. For wavelet transform, the MATLAB wavelet toolbox has been used, where only the mother wavelet, the levels of decomposition, and the option of hard or soft thresholding have to be chosen.

![Figure 2. Chromatogram for arsenic concentrations of 0.1 ug mL\(^{-1}\)](image)

4. Results and discussion

The four arsenic species are well separated with peak maxima of 4.1 min (ASA), 11.9 min (NIT) and 16.7 min (ROX). The overall measurement time was 8 min, which results due to a resolution of 0.1 second per one data point (Fig. 2).

<table>
<thead>
<tr>
<th>Species</th>
<th>Original</th>
<th>Gaussian</th>
<th>FT</th>
<th>WT(db8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASA</td>
<td>58.28</td>
<td>26.56</td>
<td>30.18</td>
<td>24.00</td>
</tr>
<tr>
<td>NIT</td>
<td>128.05</td>
<td>58.09</td>
<td>66.00</td>
<td>52.74</td>
</tr>
<tr>
<td>ROX</td>
<td>74.23</td>
<td>33.83</td>
<td>38.44</td>
<td>30.57</td>
</tr>
</tbody>
</table>

Unit: ng mL\(^{-1}\)

The peaks are clearly visible, but especially when the concentration decreases, they can hardly be distinguished from noise. Particularly applying baselines in order to calculate peak heights more precisely (and areas if required) is complicated due to noise. The limits of detection for the three arsenic species are between 24.00 and 128.05 ng mL\(^{-1}\) (Table 2).

These values should be improved, first of all by convolving the chromatograms with Gaussian distribution curves. In order to choose the best functions for convolution, the peaks of the arsenic species have to be examined and their halfwidths have to be calculated. At an arsenic concentration of 0.1 ug mL\(^{-1}\), the half-widths are 0.164, 0.258, and 0.358 min for ASA, NIT, and ROX, respectively. That means the peaks of the three organic species are all of the
different shape, the ASA peak is narrower, the ROX peak is the broadest of the three species (compare Fig. 2).

In the convolving procedure, we found that the parameter $\sigma$ of the Gaussian distribution curve can influence the SNR significantly. Take the ASA peak at a concentration of 0.1 $\mu$g mL$^{-1}$ for example. If the ratio $w_{0.5}$ of the half-widths of Gaussian distribution curve and the ASA peak is too small, the signal-to-noise ratio is not increased. However, the peak itself is heightened, but that is also true for the noise. If $w_{0.5}$ is too large, the SNR is increased in comparison to the original chromatogram as it is clearly de-noised. But the broader the Gaussian distribution curves are, the ASA peaks are also broadened too much; their peak heights are decreased. An optimum can be found for $w_{0.5} = 60\%$, as already mentioned in the literature [23]. However, this ratio may also be tolerated from 40% to 150%.

![Figure 3](image3.png)

**Figure 3.** Chromatogram for arsenic concentrations of 0.1 ug mL$^{-1}$ after convolution with a Gaussian distribution curve whose half-widths was of 0.098 min (for ASA), 0.155 min (for NIT), and 0.215 min (for ROX)

![Figure 4](image4.png)

**Figure 4.** Chromatogram for As concentrations of 0.1 ug mL$^{-1}$, de-noised by FT using a cutoff frequency of $\nu_0=200$

For the chromatograms in this work, three different Gaussian distribution curves are chosen for de-noising, with half-widths of 0.098 min (for ASA), 0.155 min (for NIT), and 0.215 min (for ROX). After the convolution, noise in the chromatograms is clearly diminished (Fig. 3), but some still remains. The peak heights are preserved relatively well; thus, the SNRs are increased. Thereby, the limits of detection are improved as well, to values in the range of 26.56-58.09 ng mL$^{-1}$ (Table 2). Furthermore, applying baselines should not be a problem anymore as the peaks can be evaluated more precisely. These results should be compared with the compatibility of Fourier transform for de-noising in the next step. For this technique, the optimal cutoff frequency $\nu_0$ has to be found. In this case, with too great values of $\nu_0$, the
chromatograms are not de-noised enough, but the peak heights are preserved. On the other hand, if the values are too small, noise is diminished, but the peaks are also flattened. There is a special problem with FT. Due to the used sine and cosine waves, the peaks may be flattened so much that the resolution is not sufficient anymore and the peaks will be overlapping (Fig. 4).

We found that the optimal cutoff frequencies are about 200 for all the three species. This relates to the fact that for the highest frequency, there are about 1.5 to 2.0 full oscillations of the sine or cosine wave within the half-width of the respective arsenic peak. Higher frequency compounds are obviously describing noise basically. De-noising with FT improves the limits of detection worse than Gaussian distribution curves. The values of the LODs are only between 30.18 and 66.00 ng mL−1 afterwards (Table 2).

As wavelet transform is generally seen as an advanced method, even better results are expected with this method. For this, some optimal “parameters” have to be found as well. The most important one is the mother wavelet that is used. As shown in Fig. 5 to Fig. 7 and Table 3, comparing different Symlets, Coiflets, as well as Daubechies wavelets, Daubechies8, whose LOD is the best one, has been found to be a very adequate one for these chromatograms due to its shape. Furthermore, hard thresholding was applied and the decomposition was carried out on six levels.

After WT, one can already see in the chromatograms (Fig. 6) that the peak heights are preserved very well, whereas the noise is diminished more than after FT and convolution with Gaussian distribution curves. The LODs for the three arsenic species are improved to values between 24.00 and 52.74 ng mL−1 (Table 3).

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Figure 7. Chromatogram for As concentrations of 0.1 ug mL\textsuperscript{-1}, de-noised by Symlets8

Table 3. LODs for the different arsenic species before and after signal treatment by three different wavelets

<table>
<thead>
<tr>
<th>Species</th>
<th>Original</th>
<th>Coif5</th>
<th>Sym8</th>
<th>Db8</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASA</td>
<td>58.28</td>
<td>29.91</td>
<td>24.81</td>
<td>24.00</td>
</tr>
<tr>
<td>NIT</td>
<td>128.05</td>
<td>65.84</td>
<td>54.50</td>
<td>52.74</td>
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<tr>
<td>ROX</td>
<td>74.23</td>
<td>38.12</td>
<td>31.60</td>
<td>30.57</td>
</tr>
</tbody>
</table>

Unit: ng mL\textsuperscript{-1}

Figure 8. Signal-to-noise ratios for As concentrations of 0.1ug mL\textsuperscript{-1} in the original chromatograms (black), after convolution with Gaussian distribution curves (red), after de-noising with FT (yellow), and with WT (green)

Comparing the three methods of signal treatment, the most important result is that all may be used for de-noising very well. Regarding for example the signal-to-noise ratios of peaks corresponding to an As concentration of 0.1 ug mL\textsuperscript{-1} (Fig. 8), it is clear that all values are increased in comparison to the respective original one (black). However, there is also a remarkable step from convolution with Gaussian distribution curves (red) via FT (yellow) to WT (green), which shows the best improvements.

These results can also be generalized by comparing the LODs. Therefore, factors of improvement compared with the original values are calculated. The average factors (as the average for all three arsenic species) are 2.2 after convolution with Gaussian distribution curves, 1.9 for Fourier transform, and 2.43 for wavelet transform. That means that after applying the first and simplest method, the LODs are already improved quite remarkably.

5. Conclusion
Due to the different toxicities and bioavailabilities of different arsenic species, it is often required not only to measure the total arsenic concentration but also those of the species separately. This was performed by HPLC-UV-HG-AFS, by which the peaks of the inorganic forms of the organoarsenic species (ASA, NIT, ROX) could be separated. However, the occurring noise in the chromatograms hinders the peak evaluation and worsens the limit of detection. Therefore, methods of signal treatment may be utilized. Three techniques-convolution with Gaussian distribution curves, Fourier transform, and wavelet transform-have been exercised and compared. In the first case, the fact could be proved that the halfwidth of Gaussian distribution curves used should only be 60% of the half-width of the corresponding arsenic peak. All three methods result in de-noised chromatograms, as well as in enlarged signal-to-noise ratios and in improved limits of detection. But of course, differences may be realized. As peaks are always flattened a little and some noise remains after convolving chromatograms with Gaussian distribution curves, they show the least capability of de-noising. However, even with this technique, limits of detection are improved by factors of about 2.2. Fourier transform is even a worse option. The best is wavelet transform, though. After de-noising the chromatograms with Daubechies8, the limits of detection are reduced by factors of more than 2.4.

Therefore, wavelet transform is proposed as the most effective method of HPLC-HG-AFS signal treatment on the basis of this research. Thus, the detection power of the analytical method is improved and the evaluation of peaks can be performed more comfortably and precisely. These results may also be transferred to other analytical methods with similar signal curves as signal treatment is bound to shape rather than to individual methods.

References
[5] Reyes Sierra-Alvarez, Methanogenic inhibition by roxarsone (4-hydroxy-3-nitrophenylarsonic acid) and related aromatic arsenic compounds , J. Hazard. Mater., 2010, 175, 352-358