

# The determination of phenazone in blood plasma for obtained sistem suitable test of monitoring drug level

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## ABSTRACT

The determining of Phenazone to human blood plasma from healthy man after separated by solid phase extraction (SPE) and spectroscopic measurements has been investigated. The objective of that research was to obtain system suitable test for determine the Phenazone level in biological fluids (human blood plasma), for new performed dosage regimented in clinical dentistry. The method can be divided into the following four steps. 1. Centrifugation the blood sample, 2. Extraction from blood plasma and, 3. Separation by SPE with manual pressured, 4. Elution to SPE followed by the measurement on a spectrophotometer in the ultra violet region. The critical value of  $|t|$  at the 5% confidence level indicates that there is no systematic error in the linearity proposed method. Recoveries for this research were obtained at ranging 93.460 to 95.598%. The coefficient variation precision of this procedure was clearly good at smallest than 2%. The analytical procedure can be carried out in one working operation as a monitored therapeutic activity.

**Key words:** phenazone, solid phase extraction, spectrophotometer UV-Vis

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## INTRODUCTION

Phenazone (as an analgesic and antipyretic properties) has been given by mouth.<sup>1</sup> Topically, solution containing 5 % of Phenazone have been used locally as ulcers drops in disorders such as acute gingivitis.<sup>2</sup> The dentist was usually approved with that dosage form as an analgesic and anti-inflammatory agent. In clinical cases, the re-arrangement of regimentation dosage of Phenazone for treated pediatrics patient was usually problems. Those problems were appeared after showed that Phenazone at long period's usage can be induced Reye syndrome of red blood cell. That problem would be reduced if the dentistry were using re-designing dosage form concepts by monitored drug level during the therapeutic periods of pediatric patients for obtained satisfaction dosage form. For obtained a suitable drug levels, the method of drug level determination was usually researching. During recent years several papers have been published on the Phenazone.<sup>3-7</sup> However, the results obtained are often in poor agreement. The accuracy and the precision of the analytical method used may give the contribution on the variation of the result. The new method of Nuraini *et al.*,<sup>8</sup> was reported that recoveries was obtained at ranging 86.010 to 88.069% and their coefficient variation of precision at ranging 0.433 to 3.871%. The recovery of new method at above was not satisfaction yet, although their precision was nearly exactly. Therefore, the purpose of this work was to develop an accurate method for the determination of Phenazone in human blood plasma using fourth-step sample preparation method.

## MATERIAL AND METHODS

The analytical method used in the present work incorporates a partition-adsorption of drug-biological matrix separation concepts.<sup>9</sup> The separation apparatus of that equipment was used Solid Phase Extraction ODS (-CH<sub>17</sub>-CH<sub>3</sub>) reverse phase at 3 ml capacity (Sigma Chem. Corp.). Plastic pestil Of 3 ml SPE ODS-C18 was used as a manual mobile phase pressure. A pure Phenazone from Sigma Corp. Chem., was used as reference drug material. The Spectronic Hitachi 1100 UV-Vis was used as a measurement drug concentration. The healthy human blood plasma (Indonesian man) was used as an artificial human blood plasma. The human blood plasma was obtained from Indonesia Red Cross Unit. The research protocol was used four step procedures as follows; the 1<sup>st</sup> step was analyzed linearity of Phenazone in distilled water from stock solution. The standard stock solutions were made up as 1000 µg.ml<sup>-1</sup> from 100 mg of Phenazone pure. The standard solutions of 0.5 to 100 µg.ml<sup>-1</sup> were prepared by sequential dilution of the standard stock solution. Both standard and stock solutions were placed in test tube. The linearity was analysis during two hours from 0.5 to 100 µg.ml<sup>-1</sup> of the standard solution vs. absorbance (Å) on 230 nm at five times replications. The result data of the step one on graphic illustration of the concentration solution series vs. absorbance unit full scale (AUF) will be used as an additional standard solution data.<sup>10</sup> The 2<sup>nd</sup> step was produced artificial samples in human blood plasma. The pure of Phenazone were weighing of 100 mg and dissolved

with human blood plasma to produce samples stock at 1000 ppm. The stock samples were prepared at ranging series additional standard concentration of 0.5-100 ppm.<sup>10-12</sup> The 3<sup>rd</sup> step was prepared artificial sample. The samples were extracted by SPE as described Akira *et al.*,<sup>13</sup> and Nuraini *et al.*,<sup>8</sup> as follows; one ml plasma samples were added 1 ml chloroform and shake up well at 10 minutes. Their solutions were added 2 ml with distilled water and centrifuge 3000 rpm (15 minutes). The supernatant were removed sample vials and keep on dark room about 5 minutes. The SPE were activated with inserting 1 ml methanol and 1 ml distilled water consists of 5% glacial acetic acid. The supernatant were inserted to SPE and vacuum drying the SPE at about 30 minutes. The SPE were added with 3 ml elution solution at rate 0.05 ml.second<sup>-1</sup> and pressured gentle with plastic pestil. Filtrate from elution were drying by N<sub>2</sub> gas and added 3 ml distilled water and analyzing to obtained absorbance values at maximum wave length (230 nm). The graphic of the additional standard solution series vs. absorbance was using for calculation drug concentration on human blood plasma after plotted their absorbance values to the graph. The Vx0 equation was used for measurement of linearity at series 0,5 to 100 ppm. The Vx0 (*Vervahrenvariationkoeffizient*) equation was described at equation 1 with Sx0 (*vervahrenstandardabweichung*) and Sy (mean residual deviation of the function) referred to equation 2.<sup>14</sup>

$$\text{Equation 1. } V \times 0 = \frac{S \times 0}{X} \bullet 100\%$$

$$\text{Equation 2, where } S_y = \sqrt{\frac{\sum (Y_i - \bar{Y})^2}{N - 2}} \text{ for } Y_i = a + bX_i$$

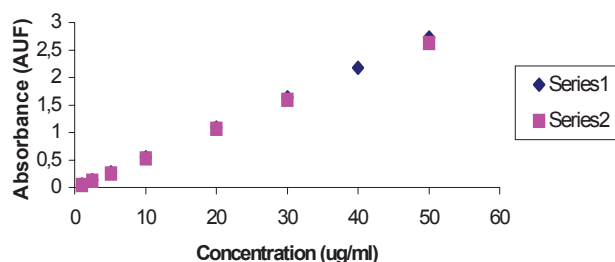
$$S \times 0 = \frac{S_y}{b}$$

In order to investigate accuracy and precision of the method the samples were spiked with standard solutions

and then analyzed by the mentioned method at below. The accuracy expressed as percent recovery was obtained by comparing the results between the Phenazone found and the Phenazone standard.<sup>15</sup> The precision expressed as percent coefficient variation was obtained by divided the values of standard deviation with the mean of concentration at five times replicates.<sup>16</sup>

## RESULT

The result procedures at the first step was appeared good correlation at ranging of the mean concentrations 0.5 to 100  $\mu\text{g.ml}^{-1}$  ( $p < 0.05$ , Table 1, Figure 1).



**Figure 1.** The linearity analysis of Phenazone dissolved in distilled water (◆) at mean of 1.002 to 50.002  $\mu\text{g.ml}^{-1}$  of 0.502 to 100  $\mu\text{g.ml}^{-1}$ . The linearity analysis of Phenazone series at 1.002 to 50.002  $\mu\text{g.ml}^{-1}$  in human blood plasma (■).

But the best purposed for additional standard was obtained at 1 to 50  $\mu\text{g.ml}^{-1}$  (CV of absorbency < 5%, Table 2 and Table 3). The V x 0 analysis of serial concentration (0.5–100 ppm) was not necessary linear (various) as referred to table 2 at below. The best serial concentration was obtained at 1.002  $\mu\text{g.ml}^{-1}$  to 50.001  $\mu\text{g.ml}^{-1}$ . The result research of accuracy and precision of that procedures were

**Table 1.** The result analysis of linearity Phenazone in distilled water

Concentration in $\mu\text{g.ml}^{-1}$					Absorbance ( $\text{\AA}$ ) in AUF					Mean $\pm$ (%CV)	
n-1	n-2	n-3	n-4	n-5	n-1	n-2	n-3	n-4	n-5		
0.502	0.501	0.501	0.504	0.505	0.503 $\pm$ 0.36	0.002	0.003	0.002	0.003	0.002	0.002 $\pm$ 22.83
1.002	1.002	1.003	1.002	1.002	1.002 $\pm$ 0.045	0.048	0.049	0.051	0.052	0.051	0.050 $\pm$ 3.273
2.504	2.503	2.503	2.504	2.503	2.503 $\pm$ 0.023	0.136	0.137	0.136	0.137	0.135	0.136 $\pm$ 0.615
5.103	5.102	5.103	5.102	5.102	5.102 $\pm$ 0.022	0.271	0.270	0.269	0.270	0.271	0.270 $\pm$ 0.310
10.003	10.002	10.002	10.003	10.002	10.002 $\pm$ 0.005	0.545	0.546	0.544	0.545	0.543	0.545 $\pm$ 0.209
20.002	20.002	20.001	20.002	20.002	20.002 $\pm$ 0.002	1.091	1.091	1.090	1.091	1.091	1.091 $\pm$ 0.041
30.002	30.001	30.001	30.002	30.001	30.001 $\pm$ 0.002	1.638	1.637	1.638	1.637	1.638	1.638 $\pm$ 0.033
40.001	40.001	40.001	40.002	40.001	40.001 $\pm$ 0.001	2.180	2.181	2.180	2.182	2.181	2.181 $\pm$ 0.038
50.001	50.002	50.001	50.002	50.001	50.001 $\pm$ 0.001	2.728	2.729	2.726	2.727	2.726	2.727 $\pm$ 0.048
60.001	60.001	60.001	59.999	60.002	60.001 $\pm$ 0.002	3.268	3.272	3.269	3.888	2.865	3.112 $\pm$ 6.293
70.001	70.001	70.001	70.001	70.000	70.001 $\pm$ 0.001	3.789	3.798	3.811	2.989	3.790	3.635 $\pm$ 9.945
80.000	80.001	80.000	80.000	80.002	80.001 $\pm$ 0.001	4.365	4.333	3.994	3.345	3.911	3.990 $\pm$ 10.336
90.000	90.002	90.000	90.001	90.002	90.001 $\pm$ 0.001	4.911	4.912	4.231	3.881	4.001	4.387 $\pm$ 11.280
100.000	100.001	100.002	100.001	100.000	100.000 $\pm$ 0.001	5.214	5.111	4.895	4.021	4.233	4.695 $\pm$ 11.421

**Table 2.** The Linear analysis of Phenazone in distilled water

The mean of serial Conct. ( $\mu\text{g}\cdot\text{ml}^{-1}$ )	Sy ( $\mu\text{g}\cdot\text{ml}^{-1}$ )	Coefficient Correlation* (r)	V <sub>x0</sub> ** (%)	X <sub>p</sub> *** ( $\mu\text{g}\cdot\text{ml}^{-1}$ )
0.503-100.00	0.446	0.971, p < 0.05 (Good)	22.431 (Not good)	33.534 (Not good)
0.503-90.001	0.099	0.998, p < 0.05 (Good)	5.585 (Not good)	7.622 (Not good)
0.503-80.001	0.076	0.999, p < 0.05 (Good)	4.841 (Moderate)	5.834 (Not good)
0.5 to 70.001	0.050	0.999, p < 0.05 (Good)	3.627 (Moderate)	3.802 (Not good)
0.5 to 60.001	0.045	0.999, p < 0.05 (Good)	3.830 (Moderate)	3.412 (Not good)
0.5 to 50.001	0.008	0.999, p < 0.05 (Good)	0.809 (Good)	0.599 (Not good)
0.5 to 40.001	0.008	0.999, p < 0.05 (Good)	1.102 (Good)	0.650 (Not good)
0.5 to 30.001	0.008	0.999, p < 0.05 (Good)	1.579 (Good)	0.706 (Not good)
1.002-100.00	0.132	0.997, p < 0.05 (Good)	6.372 (Not good)	10.656 (Not good)
1.002-90.001	0.100	0.998, p < 0.05 (Good)	5.241 (Not good)	7.917 (Not good)
1.002-80.001	0.077	0.999, p < 0.05 (Good)	4.528 (Moderate)	6.107 (Not good)
1.002-70.001	0.051	0.999, p < 0.05 (Good)	3.355 (Moderate)	3.989 (Not good)
1.002-60.001	0.046	0.999, p < 0.05 (Good)	3.557 (Moderate)	3.651 (Not good)
1.002-50.001	0.003	0.999, p < 0.05 (Good)	0.272 (Good)	0.238 (Good)

\* Good: r observation > r table at significance 5%

\*\* Not good: at > 5%, moderate: at ranging 2–5%, good: at < 5%.

\*\*\* Not good: at more than the lowest conc. of their series, good: at least than the lowest conc. of their series.

**Table 3.** Analysis of accuracy and precision of procedure determination of Phenazone in human blood plasma

Phenazone in distilled water		Absorbance ( $\text{\AA}$ ) Phenazone in blood plasma (AUF)										Intraday precision (% CV)
Drug conc. ( $\mu\text{g}\cdot\text{ml}^{-1}$ )	Absorbance (AUF)	Replication-1		Replication-2		Replication-3		Replication-4		Replication-5		
		( $\text{\AA}$ ) AUF	Recovery %	( $\text{\AA}$ ) AUF	Recovery %	( $\text{\AA}$ ) AUF	Recovery %	( $\text{\AA}$ ) AUF	Recovery %	( $\text{\AA}$ ) AUF	Recovery %	
1.002	0.050	0.043	86.000	0.047	94.000	0.041	82.000	0.042	84.000	0.046	92.000	5.913
2.503	0.136	0.127	92.029	0.125	91.912	0.123	90.441	0.128	94.118	0.126	92.647	1.508
5.102	0.270	0.259	95.926	0.257	95.185	0.255	94.444	0.258	95.555	0.256	94.815	0.615
10.002	0.545	0.528	96.887	0.527	96.697	0.525	96.330	0.524	96.147	0.521	95.596	0.522
20.002	1.091	1.067	97.800	1.066	97.708	1.063	97.433	1.061	97.250	1.062	97.342	0.243
30.012	1.638	1.601	97.741	1.600	97.680	1.597	97.497	1.598	97.558	1.596	97.436	0.116
50.002	2.727	2.616	95.929	2.618	96.003	2.620	96.076	2.622	96.150	2.630	96.443	0.206
Recovery (mean $\pm$ % CV)		94.616 $\pm$ 4.514		95.598 $\pm$ 2.197		93.460 $\pm$ 5.998		94.397 $\pm$ 5.002		95.183 $\pm$ 2.278		0.862

apparently good at mean 94.651 percent of recoveries and 0.862 percent of coefficient variation as illustrated at Table 3.

## DISCUSSION

The method described here is based partly on the method development by Nuraini *et al.*,<sup>8</sup> but by pressuring of SPE during the elution process, It was make a good recovery in determination of Phenazone in human blood plasma.<sup>17</sup>

Our recovery was apparently 1.5 to 3% highest than Nuraini *et al.*,<sup>8</sup> method at ranging 93.460 to 95.598%. The bias of precision described here was shown lowest than their protocol, although we saw both of them still satisfaction (The CV < 2%).

Each step in the assay method has been examined to give optimal results, provided the following precautions are observed. Separation by SPE should be carried out at room temperature by methanol: glacial acetic acid 0.5% (v/v) dissolve in distilled water 2 : 80 for at least 0.05 ml.second<sup>-1</sup> rate pressure to ensure complete separation of Phenazone. After drying by N<sub>2</sub>, all samples must be added by distilled water not more than 15 minutes. That process was doing to kept the chance of pH solution from acid to basic condition. The wavelength of 230 nm will be maximum detecting to aqueous sample in acid condition (5 to 6 pH solution) at not more than 2 hours.<sup>4,8</sup> Accurate timing and adequate mixing between the addition of each reagent is essential. If these precautions are taken, then reproducibility between experiments is 2%, and it is possible to determine accurately of 1 µg.ml<sup>-1</sup> to 50 µg.ml<sup>-1</sup> samples (Figure 1). The sensitivity of the method is limited at least 1 to 50 µg.ml<sup>-1</sup> sample concentration, because of a decrease in the yield of Phenazone during the sample preparation especially in pH factor.

Our method could be of use in monitoring drug concentrations in human undergoing treatment of Phenazone. Phenazone as an analgesic and anti-inflammatory purposed or for dentistry cases was available effective in human blood bodies at about 250 µg.ml<sup>-1</sup> to 500 µg.ml<sup>-1</sup>. That ranging concentration at above can be monitored by our procedures after diluted 10 times for reduced their concentration up to 50 µg.ml<sup>-1</sup> as mentioned at additional standard procedure (Table 1).

These procedures could be of use for monitored therapeutics of Phenazone for obtained suitable drug level at ranging 1 to 50 µg.ml<sup>-1</sup>. At 1 to 50 µg.ml<sup>-1</sup> of Phenazone have small deviation values of accuracy-precise and also still remain on the right tract of the recommended values of accuracy (80–120%) and precise (<10–20%). Recommended of this research was follows; at more than 50 µg.ml<sup>-1</sup>, the series concentrations must be diluted to 10 times or 100 times. The monitored therapeutic activities may be important contributors to the re-arrangement regimentation dosage, especially for dentistry in children cases or elderly cases.

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