A Portable Reflective Absorbance Spectrophotometric Smartphone

Device for Rapid and Highly Accurate Determination of Amlodipine in Pharmaceutical Formulation and Human Urine Sample

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Abstract

The simple reflective absorbance spectrophotometric smartphone device for point-of-monitoring amlodipine is presented here for the first time. The immediate analysis of amlodipine in the human urine of the patients who suffered severe side effects of this drug is very important for diagnosis, treatment, and reduction of the death rate. This measurement technique is based on the charge-transfer complex between amlodipine and picric acid, which forms a yellow product. This product can absorb light intensity from an LED strip and measure through the Blue channel from RGB mode with a smartphone application. The linear relationship for amlodipine monitoring was found in a wide range from 100.0 µg L⁻¹ to 140.0 mg L⁻¹ (R² = 0.999), and the limit of detection was found to be 25.0 µg L⁻¹. Our proposed method can be applied to the different smartphone brands with consistent sensitivity of amlodipine detection. Additionally, the determination of amlodipine in pharmaceutical formulation and human urine samples was demonstrated by our proposed method. The recoveries were indicated in the range 98.60-100.00%, which is at the acceptable level for pharmacy. This method offers interweave of basic technology and chemical analysis with environmentally friendly due to reducing the complex instrument and the amount of organic waste compared to chromatographic technique and efficient use for the detection of amlodipine. Hence, this method can be applied for promptly medical diagnoses and laboratories with limited budget resources.

Keywords: Smartphone system, Spectrophotometry, Amlodipine, Pharmaceutical formulation, Human urine
Introduction

Hypertension (high blood pressure) is one of the most common health problems owing to the most important contributors to heart disease and stroke, which together make up the world’s number one cause of premature death and disability. The World Health Organization (WHO) indicates that hypertension contributes to closely 9.4 million deaths from cardiovascular disease each year around the world. The risk of hypertension is also a major influence on cardiovascular, osteoarthritis, and kidney disease.\textsuperscript{1,2} Normally, high blood pressure treatment is the use of effective drugs to control blood pressure levels. Currently, a variety of antihypertensive drugs have been used, such as block-blockers, angiotensin-converting inhibitors, angiotensin II receptors, antagonists, diuretics, and calcium channel blockers.\textsuperscript{3} However, many antihypertensive drugs have a dose tolerance profile. Therefore, increasing the drug dose can be influential to the risk of adverse events.\textsuperscript{4} Amlodipine, which is a dihydropyridine, is widely used as the most potential antihypertensive drug owing to the food and drug administration in the United States (FDA) acceptance.\textsuperscript{5} The amlodipine is changed in the liver and released through the urinary system; meanwhile, they can be used in the human metabolism for 30-50 hours and found in human urine when taking their overdose. For pharmaceutical and clinical industries, amlodipine is produced in 2 concentrations as 5.0 and 10.0 mg per tablet because the over amlodipine dosage acquisition can cause to undesired effect for patients. It is thus essential to high accuracy quality control in the production process. Generally, the primary side effect for the use of them is peripheral edema and gingival enlargement.\textsuperscript{6-8} Nevertheless, the Ramathibodi poison center, Thailand, reported that 6 cases had been dead by overdose amlodipine.\textsuperscript{9} Thus, the prompt diagnosis of amlodipine’s side effects is so very dominant for patients to protect and treat from occurring symptoms and
reduction of the death rate. A standard method for the amlodipine analysis hasn’t been reported until now, which the developed methods for the amlodipine quantification were used in industries.

Recently, many methods for the amlodipine measurement have been reported such as spectrophotometry,\textsuperscript{10,11} high-performance liquid chromatography; HPLC,\textsuperscript{12,13} liquid chromatography–mass spectrometry; LC-MS,\textsuperscript{14,15} electrochemical sensors\textsuperscript{16,17} and spectrofluorometric method.\textsuperscript{18} Although these methods provided high sensitivity and selectivity, they also required the expensive tools, long preparation and analysis times, and large reagent consumption. Most of these approaches, the spectrophotometric method has been applied to detect amlodipine due to the simple facilitate tools, easy to processing, and low-cost analysis when compared to other instruments. For instants, R. Badran et all., presented the amlodipine determination is based on a charge-transfer complex with a cresol red reagent, which showed a yellow-orange color product in the organic phase.\textsuperscript{10} Their result exhibited high sensitivity with low limit detection as 0.071 mg L\textsuperscript{-1}; however, this process needed to perform the extraction process with chloroform affecting long analysis time. In the same way, V. Patil et all., reported the use of ninhydrin for amlodipine detection in the presence of sodium bicarbonate, which illustrated a purple color of the charge-transfer complex of them.\textsuperscript{19} Even though their consequence provided high accuracy for pharmaceutical analysis with recovery range from 90.07-105.55% but it is necessary to pretreat at a high temperature about 90.0 °C for 15.0 min. Hence, it isn’t suitable for point of care diagnosis. From both of the literature reviews, it can be seen that the charge-transfer complex principle is suitable to apply for amlodipine analysis. For the
above purpose, it is a challenge for the immediate developing method for amlodipine measurement with easy to operation, convenience, and low-cost.

Nowadays, the spectrophotometric method can be performed by using smartphone systems to reduce expensive cost, easy to use because their operating system is the smallest and portable.\textsuperscript{20-24} As well, its camera lenses are high-quality hardware to evaluate colorimetric or light intensity via Complementary Metal Oxide Semiconductor (CMOS).\textsuperscript{25,26} For instance, M. Á. Aguirre et al., developed the smartphone application for ibuprofen analysis in commercial tablets.\textsuperscript{27} Their process was based on the formation of the blue complex from the target drug and cobalt chloride. Afterward, the blue complex was extracted through dispersive liquid-liquid microextraction (DLLME) with chloroform. Additionally, they designed to apply the transmission, reflection, intensity (TRI)-analyzer conjunction with the smartphone system for final product detection through transmission and reflection of light principle. As a result, their method provided high sensitivity with a detection limit as 4.0 mg L\textsuperscript{-1} and validated recoveries as 97-105\%. Nonetheless, this approach provided some drawbacks, such as the requirement of many materials for the fabrication of a TRI-smartphone device, including mirror, laser power circuit, diffraction grating, and bifurcated fiber. Moreover, it needed the extraction procedure relative to the requirement of the long analysis time and expensive tools. Aforementioned, to develop a method for on-site analysis with inexpensive tools and to reduce analysis time from the extraction or/and pretreatment process at high temperature. Herein, we firstly reported the reflective absorbance spectrophotometric smartphone-based system for the determination of amlodipine. The developed device was designed and built with commercial material to reduce cost analysis and maintenance fees. The
quantitative analysis procedure is based on the reflective light absorption ability of the product from initial light intensity depended on amlodipine concentration and is also evaluated via the application on smartphone.28-30

In this research, the amount of amlodipine was measured by the occurring a yellow product from the charge-transfer complex by the electron transfer of the primary amine group of amlodipine to picric acid (2,4,6-trinitrophenol), which can absorb blue intensity from initial light in the model 3D chamber consisting of smartphone and cuvette. Picric acid was applied in this work because it is not necessary to use the external pretreatment process for charge transfer complex with amlodipine. Thus, our method provided many benefits, including simplicity, cost-effectiveness, low reagent consumption, rapidity, and portability. Moreover, it can be applied for the quantitative analysis of amlodipine in real samples with satisfactory precision and accuracy.

**Experimental**

See Supporting Information for descriptions of the Chemicals.

**Fabrication and apparatus**

To create suitable reflective absorbance spectrophotometric equipment for analysis, a black acrylic chamber chose to disrupt reflective light from the white background as the dimensions as 6.0 x 22 x 6.5 cm (width, length, and height) was designed as model 3D consisting of LED strips (white light, 2.4-Watt constant, World semi) for a light controller. The instrument was built as a closed system with constant light intensity, in which the background of the instrument's interior, opposite to LED strips and a smartphone camera, as white to reduce the light absorption. Also, light intensity was
focused through the chamber's circular cavity, as indicated in Figure. 1. Color grab (Loomatix version 3.6.1) was applied as smartphone applications to detect the light intensity and display RGB data, which indicates a value in the range 0-255 in each channel. Cuvette (Quartz cell, series No 06-907) with a capacity of 4.0 mL from Coax Group Corporation Ltd. was used in the experiment. UV-Visible spectrophotometer (Shimadzu, UV2100) was performed in the experiment.

Standard solution preparation

Standard amlodipine solution (500.0 mg L⁻¹) was performed through 0.05 g amlodipine dissolved by ACN and adjusted the total volume to 100.0 mL in a volumetric flask. Picric acid solution (2500.0 mg L⁻¹) was performed through 0.25 g picric acid dissolved by DCM and adjusted the total volume to 100.0 mL in a volumetric flask. All of the interference agent solutions (2500.0 mg L⁻¹) were performed through 0.0625 g interference agents dissolved by ACN and adjusted the total volume to 25.0 mL in a volumetric flask.

Optimum conditions

Parameters such as measurement channels, solvent effect, picric acid concentration, reaction time, and stability time were studied. The experiment was tested through the sensitivity system, which slope value between analytical signal and amlodipine concentration in the range 2.0-10.0 mg L⁻¹ for all of the parameters. The product solution in the cuvette was inserted into the closed design equipment chamber. Then, the Color Grab application on the smartphone was used to measure the light intensity directly with
a non-capture image. Job’s method was studied by various volumes ratio of amlodipine and picric acid at the same concentration (0.5 µmol L⁻¹).

Analytical characteristics procedure

The calibration curve was obtained by the various concentrations of amlodipine in ACN solution from 0.10 to 140.0 mg L⁻¹ mixed with picric acid (2500.0 mg L⁻¹, 0.60 mL) in the final volume as 3.0 mL adjusted by ACN. Repeatability and reproducibility (intra-day and inter-day) were determined by analysis of amlodipine at 5.0, 10.0, 50.0 and 100.0 mg L⁻¹. The blank solution was performed by added picric acid (2500.0 mg L⁻¹, 0.60 mL) and adjusted the total volume as 3.0 mL in a cuvette.

Interference testing procedure

The interference effect was evaluated by measuring the response signal of amlodipine with different interference ratios. The experiment was conducted by adding a standard of amlodipine (500.0 mg L⁻¹, 0.60 mL) and mixing with various types of interference (5.0 g L⁻¹, 0.60 mL). Then, the picric acid (2500.0 mg L⁻¹, 0.60 mL) was added and adjusted the final mixture volume to 3.0 mL by ACN in a cuvette. Finally, the product solution was put into the developed chamber and was measure with Color Grab application on a smartphone.

Pharmaceutical formulation testing

Amlodipine, 20 tablets, has been thoroughly crushed and weighed precisely. After that, it was dissolved and adjusted to the final volume as 50.0 mL by ACN. The amlodipine solution (0.15 mL) was added into the cuvette containing picric acid (500.0 mg L⁻¹, 0.60 mL), and adjusted the total volume as 3.0 mL by ACN in a cuvette.
Results and Discussion

Principle of proposed technique and its acquisition

Our developed technique is based on a reflective light feature. Concisely, when the incident light hits to smooth material, it will reflect the original direction, which is well known as “specular reflection”. Thus, the frequency of reflected light is equal to the frequency of the incident light because the incidence angle is always like the reflection angle according to basically reflective light principle.31

In this experiment, the light from LED strips travels to the white background; after that, it reflects through a product solution in a cuvette and through a smartphone’s camera, which is installed on the same side as LED strips as indicated in Figure. 1(c). The light intensity was hence absorbed by produce solution that is proportional to the concentration of amlodipine and was detected with CMOS sensor in the smartphone because it can change light signal through electric measurement by photosite to Red-Blue-Green (RGB) mode via Color Grab application. Consequently, our approach can measure light intensity with primary color separation. Besides, we evaluated analytical signal (S) values from suitable channels which were calculated according to Eq. 1, where I is the light intensity of the final solution and I0 is the light intensity of blank solution to avoid the errors that result from obtaining images with slight variations in brightness.30

\[ S = -\log \frac{I}{I_0} \]  
(Eq. 1)

Evaluation of the analytical signal by each channel was separately performed by comparing slopes of the calibration curve generated by the concentrations of amlodipine from 2.0 to 10.0 mg L\(^{-1}\) as a result indicated in Figure. 2. Blue channel response provided
the highest sensitivity and linearity because the absorption of yellow products mostly absorbs the violet light (Blue and Red channel).\textsuperscript{32} We hence selected this channel for the next experiment. The experimental result is related to the spectrum from the UV-Visible spectrophotometer of an occurred product, as indicated in Figure. S1. It can be explained that the absorption peak of picric acid is found at 250 nm in the UV range because picric acid is colorless in the organic solvent. When the charge-transfer complex occurred, it affected to spectrum shift toward the bathochromic shift at 420 nm, which indicated the absorption peak of the yellow product.

Optimization studying

The distance between the smartphone holder and sample holder (the focusing distance) was optimized and discussed in Figure. S2 “Supplemental Information file”. Afterwards, we studied the different solvents for the preparation of amlodipine and picric acid in this assay, as a result, indicated in Figure. 3(a) and (b). Accordingly, ACN and methanol provided the great sensitivity for amlodipine preparation because it can dissolve in the middle to high polarity solvent and has a distribution coefficient as 2.76. However, methanol also offered a higher standard deviation than ACN, so we selected ACN as a suitable solvent for amlodipine.\textsuperscript{33} In the same way, DCM and chloroform showed perfectly sensitivity for picric acid preparation owing to their non-polarity dissolve ability. Still, we chose DCM as a great solvent for them to avoid toxicity from chloroform usability. The picric acid concentration was studied as a result indicated in Figure. 3(c). On the whole, the result demonstrated that increasing of picric acid concentration between 200.0-500.0 mg L\textsuperscript{-1} led to a significant increase in the sensitivity and levels off at 500.0 mg L\textsuperscript{-1}. To reduce chemical reagents, we chosen this concentration for analysis.
Afterward, analysis time influence was found to be complete for quantitative analysis when the interweave reagent and sample are mixed immediately. It demonstrated that the reaction time does not affect the signal measurement of the occurred product. For stability of the proposed method, analytical signal detection of the final product during six days was investigated. The signal didn’t change for three days, and the signal has been reduced to 92.47% from the beginning signal when the mixture solution was kept for six days.

Job’s continuous variations curve for this reaction was investigated to understand the mole ratio between amlodipine and picric acid, as indicated in Figure 3(d). The signal reached up at 0.50 of mole fraction, which referred to a 1:1 of them. Amlodipine is the n-donor electron, and picric acid is π-acceptor so that the reaction mechanism can predict from the infrastructure of both substances. Shortly, a lone-pair electron on the primary amine group of amlodipine, which serves as based-Lewis, transfers to the hydroxyl group of picric acid, which serves as acid-Lewis, via electrostatic force on behalf of a charge-transfer complex. After that, picric acid loses a proton to amlodipine in a suitable solvent occurring as picrate ion, which increased yellow intensity, as indicated in Figure. S3.

Analytical performance

The validation method for the determination of amlodipine using the proposed technique was characteristics by guideline ICH to assessed performance and validity. A linearity range for amlodipine was found in the range of 100.0 µg/L to 140.0 mg L⁻¹ with \( R^2 \) as 0.999 in Figure 4 as well as the product solution in Figure. S4. The limit of detection (LOD) calculated by 3-fold of blank signal standard deviation dividing by the slope of linearity was 25.0 µg L⁻¹. To compare with conventional spectrophotometric method, we found that proposed technique can provide the greater detectable performance.
owing to the offering detection limit lower than traditional assay (30.0 µg L⁻¹). Moreover, this developed device also shown more benefit than spectrometric instrument in term of cost-effectiveness, easy to portability and low power handing. Repeatability and reproducibility (intra-day and inter-day) were determined at four amlodipine concentration at 5.0, 10.0, 50.0 and 100.0 mg L⁻¹ (n=10) and found the highest relative standard deviation values (RSD) as 1.34% (Table S1). It indicated that the excellent stability and precision for the amlodipine monitoring under the optimum conditions in Table S2.

The comparison of analytical performance obtained by this proposed method and other methods using spectrophotometric techniques was showed in Table 1. Altogether, our proposed method exhibited a wide linearity range and the lowest detection limit for the quantitative analysis of amlodipine comparing with the other methods without the difficult sample preparation method such as the extraction step and heat pretreatment step. Furthermore, the proposed technique can be portable to on-site measurement so that it can be considered as a simple, fast and convenient method for point of care analysis and industries routine.

Interference testing

The influent interference testing was performed by measuring the analytical signal through mixing between amlodipine at 100.0 mg L⁻¹ and each interference agents, some biochemical and pharmaceutical substrate at 1.0 g L⁻¹, which was expected to be found in the real sample, as indicated in Figure. S5. There were no different signals from normally beginning signal over ±10%. Hence, no interferences were observed in the presence of all of the substances for concentration in real samples. Even though some interference
agents have an amine group similar to amlodipine such as uric acid, creatinine, and albumin but no interference signal for analysis because uric acid or creatinine can react to picric acid in an alkaline solution, likewise, albumin can react with picric acid occurring fluorescence signal that needs the exciting light in the ultraviolet range, but our method excited the spectrophotometric detection with LED as a visible light range.\textsuperscript{48-50} Similarly, some medicine content may be found in urine sample owing to their overdose and metabolism time.\textsuperscript{6,7} But there were no interferences from general drug in this assay. Thereby, amlodipine analysis in real sample with this method demonstrated noninterference although the patient will take many medicines.

Real sample analysis

The proposed smartphone system technique was applied for the quantitative analysis of amlodipine in four pharmaceutical formulation samples from local markets, as a result, indicated in Table 2. Evaluation of pharmaceutical formulation analysis in four-samples including A, B, C, and D was found and calculated as 5.04, 4.96, 10.18, and 10.09 mg/tablet, respectively. Accuracy of the analytical technique was employed by comparing the quantitative values from the proposed method and the labeled amounts via t-test calculation as 0.53-2.23 that gave no significant at 95% confidence levels ($T_{\text{critical}} = 4.30$) and RSD as 1.46-2.62%. It demonstrated the high accuracy and precision of developed technique for the amlodipine determination in drug formulation, which in the acceptable level of pharmacy.

Moreover, the validity confirmation of the proposed technique was determined by the spiking method. The pre-analyzed pharmaceutical powder was spiked with the standard of amlodipine as three different concentrations (5.0, 10.0, and 15.0 mg L$^{-1}$), and
total concentrations were determined by the proposed method as a result indicated in Table 3. By experimental result can calculate recoveries as 99.47-100.00%, and the highest RSD was about 1.16%. Thus, it demonstrated that the excipients present in the pharmaceutical formulation unaffected in the assay. Last, of all, the examination of the developed method for biological fluid samples, such as human urine, was operated with a spiking method. This assay was performed by spiked amlodipine at four different concentrations (5.0, 10.0, 15.0, and 20.0 mg L⁻¹) into the human urine sample as a result indicated in Table 4 and was examined by using the spectrophotometric method as indicated in Figure. S6. The calculated amount of amlodipine that spiked into human urine samples was 4.98, 9.95, 14.85, and 19.72 mg L⁻¹, respectively. Evaluated recoveries of the developed method for human urine samples were 98.60-99.60% without an influent organic solvent, so it can be used to analyze the quantitative amlodipine in human urine effectively.

**Conclusion**

The proposed technique offers a new strategy for smartphone-based reflective absorbance measurement with simplicity, rapidity, cost-effectiveness, low reagent consumption and waste generation, portability, high stability, accuracy, and precision for quantitative analysis of amlodipine in real samples. When compared to the previous techniques described in the literature, it demonstrated the best analytical performance. This developed method was alternatively advisable for the amlodipine determination in quality control routines and point of care for clinical analysis.

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Table 1. Comparison of analytical performance obtained by the proposed method with other spectrophotometric methods.

| Reagent        | Linearity range (mg L⁻¹) | Detection limit (mg L⁻¹) | Requirements                     | Reference |
|----------------|--------------------------|--------------------------|----------------------------------|-----------|
| Cresol Red     | 1.1-5.5                  | 0.071                    | Extraction                       | 15        |
| Ninhydrin      | 5-250                    | 0.09                     | Pretreated at 90 °C              | 24        |
| Urea           | 5-25                     | 2.0                      | -                                | 39        |
| Eosin Y        | 5-60                     | 1.8                      | Dissolved in surfactant          | 40        |
| TCNQ           | 20-110                   | 0.75                     | -                                | 41        |
| TCNE           | 5-35                     | 0.60                     | -                                |           |
| Ferricyanide   | 5-15                     | -                        | Dissolved in acid                | 42        |
| BTB            | 5-40                     | -                        | Extraction                       | 43        |
| BCP            | 4-20                     | -                        | Extraction                       | 44        |
| HCl            | 4-20                     | 0.165                    | Dissolved in acid                | 45        |
| NR             | 10-50                    | 0.132                    | -                                | 46        |
| NR             | 2-30                     | 1.24                     | Dissolved in acid                | 47        |
| Picric acid    | 0.01-140                 | 0.025                    | -                                | This work |

TCNQ = 7,7,8,8-tetracyanoquinodimethane  TCNE = tetracyanoethylene
BTB = bromothymol blue  BCP = bromocresol purple
NR = not reagent

Table 2. Result of pharmaceutical formulation samples analysis by our developed method (n=3).

| Pharmaceutical formulation | Total found (mg/tablet) | Specific label (mg/tablet) | T-test | %RSD |
|-----------------------------|-------------------------|----------------------------|--------|------|
| A                           | 5.04 ± 0.001            | 5.0                        | 0.53   | 2.58 |
| B                           | 4.96 ± 0.001            | 5.0                        | 0.53   | 2.62 |
| C                           | 10.18 ± 0.001           | 10.0                       | 2.23   | 1.46 |
| D                           | 10.09 ± 0.001           | 10.0                       | 1.11   | 1.47 |
### Table 3. Recoveries of amlodipine in pharmaceutical formulation samples by spiked method (n=3).

| Pharmaceutical formulation | Amlodipine in tablet (mg L\(^{-1}\)) | Pure amlodipine added (mg L\(^{-1}\)) | Found (mg L\(^{-1}\)) | %Recovery | %RSD  |
|----------------------------|--------------------------------------|---------------------------------------|------------------------|-----------|-------|
| A                          | 5.04                                 | 5.0                                   | 10.00                  | 99.61± 0.000 | 0.00  |
|                            |                                      | 10.0                                  | 15.01                  | 99.81± 0.002 | 1.15  |
|                            |                                      | 15.0                                  | 20.04                  | 100.00± 0.002 | 0.92  |
| B                          | 4.96                                 | 5.0                                   | 9.91                   | 99.50± 0.001 | 1.49  |
|                            |                                      | 10.0                                  | 14.91                  | 99.67± 0.002 | 1.16  |
|                            |                                      | 15.0                                  | 19.93                  | 99.85± 0.002 | 0.93  |
| C                          | 10.18                                | 5.0                                   | 15.11                  | 99.54± 0.000 | 0.00  |
|                            |                                      | 10.0                                  | 20.16                  | 99.90± 0.000 | 0.00  |
|                            |                                      | 15.0                                  | 25.16                  | 99.92± 0.002 | 0.83  |
| D                          | 10.09                                | 5.0                                   | 15.01                  | 99.47± 0.002 | 1.15  |
|                            |                                      | 10.0                                  | 20.04                  | 99.78± 0.002 | 0.92  |
|                            |                                      | 15.0                                  | 25.03                  | 99.76± 0.000 | 0.00  |

### Table 4. Recoveries of amlodipine in human urine samples by spiked method (n=3).

| Pure amlodipine added in urine sample (mg L\(^{-1}\)) | Total found (mg L\(^{-1}\)) | %Recovery |
|------------------------------------------------------|-----------------------------|-----------|
| 5.0                                                  | 4.98                        | 99.60     |
| 10.0                                                 | 9.95                        | 99.50     |
| 15.0                                                 | 14.85                       | 99.02     |
| 20.0                                                 | 19.72                       | 98.60     |
**Figure captions**

**Figure. 1** Designed device (a) reflective absorbance spectrometric smartphone systems, (b) view of the suitable device with difference focusing distance such as 5.0 cm (1), 10.0 cm (2), 15.0 cm (3) and 20.0 cm (4) and (c) principle for the reflective of light in the proposed device.

**Figure. 2** Sensitivity of different channels for the detection of amlodipine by our proposed method which [▲] blue channel, [■] red channel and [♦] green channel.

**Figure. 3** Optimum conditions of the amlodipine analysis by our proposed method including the different solvents of (a) amlodipine or (b) picric acid preparation, (c) concentration of picric acid and (d) Job’s continuous variations.

**Figure. 4** Linearity range of amlodipine in the range 100.0 μg L⁻¹-140.0 mg L⁻¹.
Figure. 2

![Graph showing the relationship between concentration of amlodipine and analytical signal for different channels.](image)

- **Red channel**
  - Equation: $y = 0.0092x + 0.0047$
  - $R^2 = 0.9989$

- **Green channel**
  - Equation: $y = 0.0060x + 0.0008$
  - $R^2 = 0.9927$

- **Blue channel**
  - Equation: $y = 0.0047x - 0.0017$
  - $R^2 = 0.9849$
Figure 3

(a) Difference solvents for amlodipine preparation

(b) Difference solvents for picric acid preparation

(c) Concentration of picric acid / mg L\(^{-1}\)

(d) Volume\(_{\text{amlodipine}}\) / Volume\(_{\text{total}}\)
Figure. 4

The graph shows a linear relationship between the concentration of amlodipine and the analytical signal. The equation of the line is:

\[ y = 0.0089x + 0.0055 \]

with an \( R^2 = 0.9999 \).
Graphical abstract