

Original Article



Stability of acetylsalicylic acid in human blood collected using volumetric absorptive microsampling (VAMS) under various drying conditions

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ABSTRACT

Acetylsalicylic acid (ASA) is one of the most commonly used medications in global market, with a risk of intoxication in certain patients. However, monitoring blood drug concentration often requires frequent hospital visits; hence there is an unmet need to increase patient-centricity by conducting blood sampling at home. Volumetric absorptive microsampling (VAMS) is a device that allows collection of homogenous and accurate volume of blood without venipuncture, and can be utilized by patients who are not in hospital settings; but because ASA is prone to hydrolysis and stabilizing reagents cannot be added to VAMS samples, a way to improve sample stability must be developed. The objective of this study was to identify the cause of instability with ASA samples collected by VAMS, and to evaluate ways to improve sample stability. A liquid chromatography with tandem mass spectrometry (LC-MS/MS) was used for analysis of ASA concentration in whole blood. Samples collected with VAMS were kept under different drying conditions (desiccator, pressurized, nitrogen gas and household vacuum sealer) and were compared to the control samples collected by conventional venous sampling. The recovery of ASA was about 31% of the control when VAMS sample was dried at room temperature, whereas VAMS samples under humidity controlled conditions showed more than 85% of recovery. Our results suggest that adequate level of humidity control was critical to ensure sample stability of ASA, and this humidity control could also be achieved at home using household vacuum sealer, thus enabling patient-centric clinical trials to be conducted.

Keywords: Blood Specimen Collection; Acetylsalicylic Acid; Stability; Patient-Centered Care

INTRODUCTION

Acetylsalicylic acid (ASA), also known as Aspirin, is among the oldest and the most frequently prescribed drugs, with an estimated 120 billion pills produced annually [1]. It is used for a wide array of diseases and conditions, including fever, mild to moderate pain and

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Conflict of Interest

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recurrent attacks of vascular disease [2]. Typical concentration of aspirin is between 10 and 30 mg/dL of blood; any concentration above 40 mg/dL can be toxic. Signs of overdose include abnormally deep or rapid breathing, fever, increased heart rate, and ringing in the ear [3]; in extreme cases, brain functions could be altered. Salicylate blood test can be conducted as an emergency test for ASA intoxication or as a routine test to monitor the drug concentration in blood; although the routine ASA concentration monitoring is unnecessary for most patients, it is important for those with rheumatoid arthritis or other autoimmune disorders[4-6]. However, blood tests are conducted mainly in hospital settings; patients have to visit outpatient clinic to receive regular check-ups. Because there is an unmet need for patients to minimize hospital visits, a way to conduct blood test and accurately measure blood drug concentration without visiting hospital is under development.

Launched in 2014, volumetric absorptive microsampling (VAMS) is a fixed volume collection device that can be used to sample blood easily. A hydrophilic tip of VAMS absorbs a specific volume of blood through capillary action and analysis is performed after drying [7,8]. Within a few seconds of finger prick at a 45° angle at fingertips, the VAMS tip can directly contact the blood droplet, and a precise volume of blood (10 µL) is collected within the hydrophilic tip. It also allows homogenous collection of the sample across the tip, overcoming hematocrit effect associated with dried blood spot (DBS) technique [9]. Since the procedure is far less invasive than the conventional venous sampling that utilizes venipuncture, patient convenience is improved, especially in pediatric patients and those with renal or hepatic diseases [10-12]. Patients can collect a certain volume of peripheral blood and dry the sample, store and transport under ambient conditions per manufacturer's brochure. Simplification of blood sampling process with the utilization of VAMS device allows more frequent analysis, and the device could be employed in various settings including preclinical toxicology, clinical pharmacokinetic studies and therapeutic drug monitoring [13].

However, limitations exist as the addition of reagents during the blood collection step is not possible; the porous hydrophilic tip of VAMS is made to absorb a specific volume of blood and, if some reagents were to be added, a desirable volume of blood would not be collected. Addition of reagents during VAMS sampling also negates the benefit of homogenous distribution across the site of collection. In conventional blood sampling to analyze ASA concentration, esterase inhibitors like potassium fluoride and sodium fluoride are added to the analytes immediately after blood collection since ASA is readily hydrolyzed to salicylic acid by plasma esterase in the absence of stabilizing reagent [14-16].

In our preliminary study [17], we have observed that recovery rates at ambient temperature and relative humidity significantly dropped to 27.4–35.5% after drying as ASA undergoes hydrolysis without addition of stabilizer. There also have been reports of unsatisfactory recovery rates of certain drug analytes, such as fosfomycin, hydroxyurea, and raltegravir, due to chemical degradation [18-20]. According to the product brochure, VAMS samplers in rack are to be dried for approximately for 3 hours under ambient conditions, then either to be returned to the collection plate and box for storage/transportation or extracted for analysis. However, the aforementioned publications about fosfomycin and other drugs had suggested samples to be frozen at -70°C due to a marked drop in sample stability, contradicting manufacturer's guideline.

In light of these previous studies, the objective of this study was to identify the cause of instability with ASA samples collected by VAMS and to evaluate ways to improve sample

stability. By experimenting different modalities of drying, transportation, and storage environment, a way of circumventing the necessity for stabilizing reagent was investigated.

METHODS

Chemicals and materials

Acetylsalicylic acid, hydrochlorothiazide, potassium fluoride and formic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). High-performance liquid chromatography (HPLC) grade acetonitrile and water were purchased from Fisher Scientific (Waltham, MA, USA). VAMS devices (Mitra[®]) were purchased from Neoteryx (Torrance, CA, USA). The chemical structures of acetylsalicylic acid and hydrochlorothiazide (as an internal standard, IS) are presented in **Fig. 1**.

Chromatographic and mass spectrometric conditions

All samples were analyzed by liquid chromatography with tandem mass spectrometry (LC-MS/MS) consisting of an Agilent Technologies HPLC 1100 series (Santa Clara, CA, USA) coupled to Applied Biosystems 4000 QTRAP (Foster City, CA, USA) mass spectrometer. Chromatographic separation was performed using a Luna, C18 column (2.0 × 50 mm, 5 μm; Phenomenex, Torrance, CA, USA) maintained at 30°C and achieved under isocratic conditions of 0.1% formic acid in water:0.1% formic acid in acetonitrile (10:90, v/v) with a flow rate of 0.2 mL/min and 5 μL of the injection volume. Quantitation was achieved by MS/MS detection in negative electrospray ionization mode and the optimized conditions are as follows: for ASA and internal standard, declustering potential was -25 and -95 V, both entrance potential was at -10 V, collision energy was -10 and -46 V, and collision cell exit potential was at -23 and -13 V, respectively. The detection of ions was performed in the MRM mode by monitoring the transition pairs of m/z 178.9 to m/z 137.0 for acetylsalicylic acid (analyte) and m/z 295.9 to m/z 78.1 for hydrochlorothiazide (IS). The dwell time for each transition was 300 ms. Peak area ratios were calculated using AB Sciex Analyst[®] 1.4 (Foster City, CA, USA) in **Fig. 2**.

Preparation of standard solutions

The primary standard stock solutions were prepared by dissolving 1 mg of ASA in 1 mL of 0.1% formic acid in 50% acetonitrile and stored at -20°C. These stock solutions were successively diluted with 0.1% formic acid in 50% acetonitrile to give concentrations of 0.4, 0.8, 1, 4, 8 and 10 μg/mL for calibration standards. The chromatographic signal-to-noise (S/N) ratio was greater than 3 at the limit of detection (LOD) and greater than 10 at the limit of quantitation (LOQ).

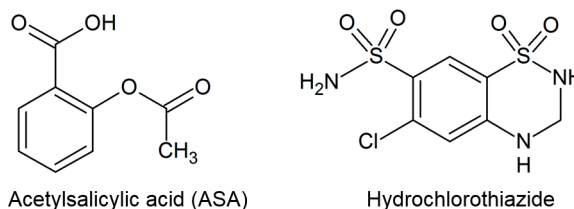


Figure 1. Chemical structures of ASA and hydrochlorothiazide as an internal standard. ASA, acetylsalicylic acid.

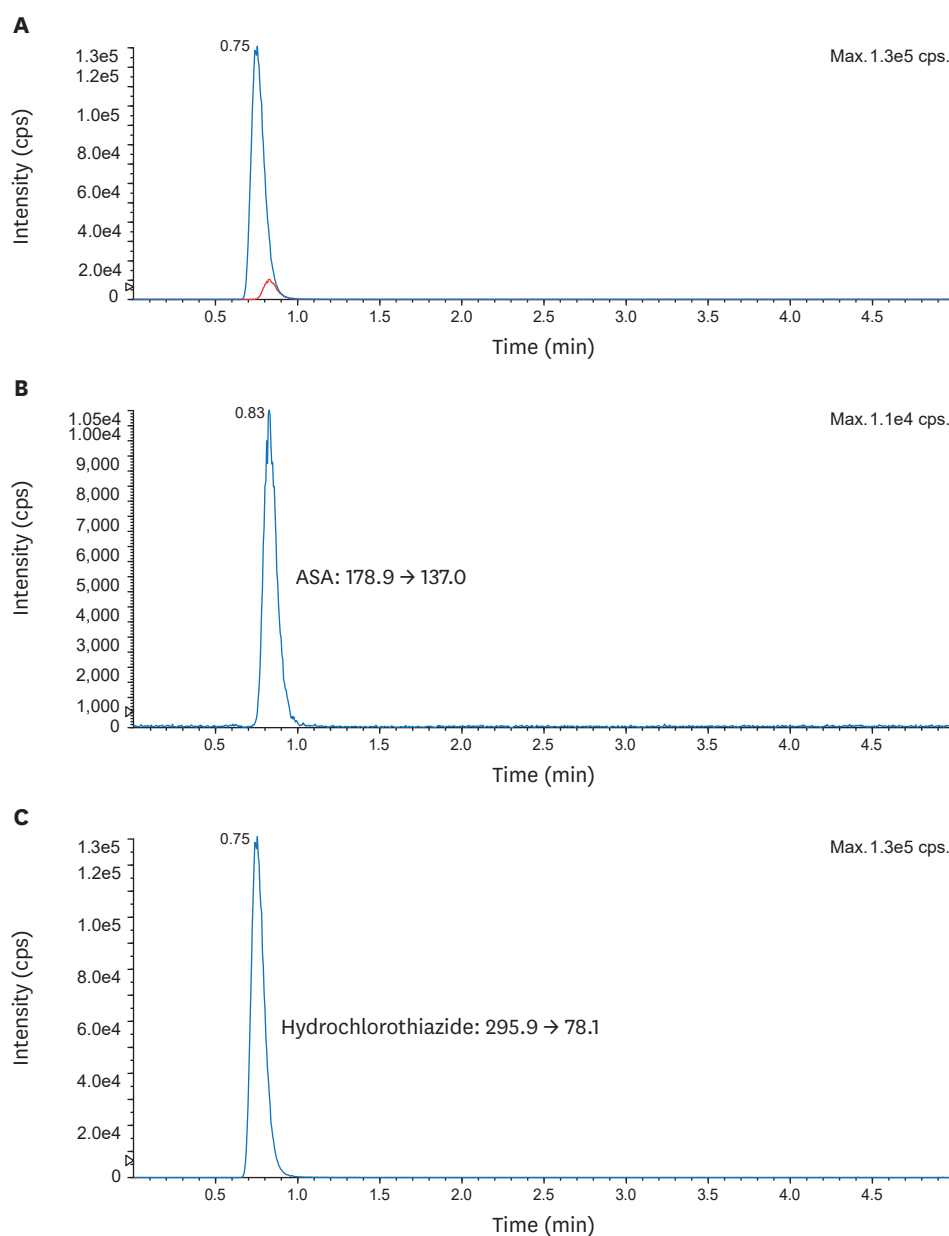


Figure 2. LC-MS/MS chromatograms of ASA and hydrochlorothiazide. (A) Double peaks of ASA and hydrochlorothiazide, (B) ASA (800 ng/mL), (C) internal standard (5 µg/mL). LC-MS/MS, liquid chromatography with tandem mass spectrometry; ASA, acetylsalicylic acid.

Quality control (QC) samples were prepared at three different levels with following concentrations: 40 (low quality control, LQC), 400 (medium quality control, MQC) and 800 (high quality control, HQC) ng/mL. The internal standard stock solution (hydrochlorothiazide, 10 µg/mL) was also prepared in 0.1% formic acid in 50% acetonitrile.

Sample preparation procedure

Whole blood samples were first collected by conventional venous blood sampling. Calibration standards were prepared within the ASA concentration range of 40–1,000 ng/mL by spiking 10% stabilizer (50% potassium fluoride in water, 10 µL) and each working standard (10 µL) in 80 µL of whole blood sample. Potassium fluoride was added to whole

blood in order to prevent enzymatic hydrolysis of ASA. To analyze the samples collected by conventional venous sampling, a 10 μ L aliquot of whole blood calibration standard was placed into an Eppendorf tube that contained 10 μ L of IS. To analyze the samples collected by VAMS, the hydrophilic tip was placed in direct contact with whole blood calibration standards for 2 seconds, and after complete absorption of ASA-containing whole blood, the tip was dried at room temperature for 1 hour. Dried VAMS tips were separated from the handle of the device and placed into Eppendorf tubes that contained 10 μ L of IS. For protein precipitation, the Eppendorf tubes were sonicated for 15 minutes in presence of 250 μ L of acetonitrile, centrifuged at 10,000 rpm for 10 minutes at 4°C, and supernatants were transferred to injection vials.

Method validation

Calibration curve

Method validation was conducted according to the Guideline on Bioanalytical Method Validation published by Korean Ministry of Food and Drug Safety (MFDS) [21]. ASA concentration in whole blood was validated over the range of 40–1,000 ng/mL. The calibration curves were generated using linear relationship between peak area of hydrochlorothiazide (IS) versus ASA (analyte) with r always larger than $R = 0.995$, weighted using $1/x$. Lower limit of quantification (LLOQ) was the lowest concentration of analyte that could be quantitatively determined with acceptable precision within $\pm 20\%$ and accuracy within 80–120%.

Precision and accuracy

Six different ASA concentrations (40, 80, 100, 400, 800 and 1,000 ng/mL) were used to assess the precision, accuracy and stability of ASA in whole blood samples. Precision and accuracy were evaluated at each level by adding stabilizer to the whole blood by five replicates within the day (intra-day precision) and between days (inter-day precision). The coefficient of variation (CV%) was used to determine the precision for each replicate by calculating $(\text{standard deviation}/\text{mean}) \times 100\%$ and considered acceptable if the values were within $\pm 15\%$ ($\pm 20\%$ at LLOQ). Accuracy was calculated as $(\text{measured concentration}/\text{nominal concentration}) \times 100\%$ and considered acceptable if the values were within 85–115% (80–120% at LLOQ).

Extraction efficiency

The protein precipitation procedure for samples collected by VAMS was as described in section 2.4. The extraction efficiency (%recovery) was calculated by comparing pre- and post-sonication concentration of ASA to evaluate if the extraction of ASA on VAMS sampling was efficient and reproducible when sonication process was added. The %recovery was calculated as $(\text{measured concentration pre- or post-sonication}/\text{initial concentration}) \times 100\%$, evaluated with triplicates at each QC concentrations of ASA.

Compatibility

ASA concentrations in samples collected by conventional venous blood sampling or by VAMS were compared to assess equivalence between sampling methods. The concentrations in both conventional venous samples and VAMS samples were calculated from the calibration curve previously made from standard solutions with spiked ASA in whole blood. The concentrations between two samples were compared in terms of mean (ng/mL), precision (CV%) and accuracy (%bias).

Stability of ASA in dried blood collected by VAMS

The stability of ASA collected by VAMS was investigated on three levels. First, the effect of stabilizer utilization was investigated as follows. The samples collected by VAMS were prepared at concentrations of QC level (40, 400 and 800 ng/mL) with or without additional stabilizer, while the control sample collected by conventional venous blood sampling was prepared by adding stabilizer. This condition was to imitate actual practice settings; blood collected by conventional means would have stabilizer added to the samples, while VAMS samples would not have any stabilizer added.

Second, because it was critical to precisely control humidity levels to prevent hydrolysis of ASA in blood samples [22], the stability of ASA dried at different conditions was investigated. The control sample was collected by conventional venous blood sampling, and after adding stabilizer, it was kept at room temperature (RT, 23°C) for one hour to imitate the drying condition of VAMS samples. Multiple VAMS samples were collected by having the VAMS tip in direct contact with whole blood, and were dried at RT or in an atmosphere with controlled relative humidity (RH) levels, for one hour. ASA stability under various conditions while drying VAMS tip was assessed by comparing the %recovery of ASA in blood dried at RT or at dehumidification conditions (11% RH).

Four different drying conditions were used to precisely control dehumidification and to compare the results. In order to achieve a low RH atmosphere within a desiccator, both desiccant-based and pressurization drying conditions were used, and the conditions were as follows. Firstly, 50 g of calcium chloride (CaCl₂) was used as the working desiccant material for dehumidification (11% RH) in desiccator (A-Desiccator with calcium chloride). The second condition utilized the introduction of pure nitrogen into the hypoxia chamber, which could replace a humid atmosphere with an inert atmosphere and could provide low-moisture conditions (B-Hypoxia chamber with nitrogen). In this condition, nitrogen-based hypoxia chamber could control RH tightly. Thirdly, pressurized desiccator set under 70 KPa using vacuum was utilized (C-Desiccator with vacuum), and the minimum RH of the compressed air within this desiccator could reach 11% RH. In addition, samples collected by VAMS were vacuum-sealed using household vacuum sealer and were dried at RT to prevent moisture exposure and hydrolysis of ASA (D-Household vacuum sealer). The last condition was set to imitate the actual practice setting at home, where VAMS might be used by patients themselves and could be sealed using household vacuum sealer. Concentration of ASA in each sample with different drying conditions compared to the control was evaluated in terms of mean (ng/mL), precision (CV%) and %recovery.

Last, in addition to the different drying conditions, long-term stability was tested by keeping the samples for one month under the same condition and then analyzing the concentration of ASA, to evaluate if the sample stability was affected after a long-term storage. All the conditions (excluding RT drying condition) were same as aforementioned conditions, except for household vacuum sealer condition (D); the long-term stability was tested after one month under RT, 4°C, and -20°C storage temperature, to imitate the condition where VAMS sampling was done at home, and samples were stored either at RT or in household refrigerator.

RESULTS

Validation of analytical method for ASA in whole blood

Calibration curves with spiked ASA in whole blood collected by conventional venous sampling were prepared, with correlation coefficients (r) ≥ 0.999 for all calibration lines. The observed results of precision ranged from 0.9–5.4% for intra-day and from 1.4–6.9% for inter-day, which were within acceptance criteria. Intra- and inter-day accuracies ranged from 91.7–109.6% and from 93.5–106.8%, respectively, which were also within acceptance criteria (Table 1).

Method optimization

While VAMS was originally designed to simplify collecting, processing, and extracting processes, a way to optimize extraction procedure was needed because a study reported that different extraction efficiency was reported before and after ultrasonic extraction of ASA [23]. Therefore, we have included sonication for 15 minutes in this study to optimize extraction. The mean extraction efficiency was increased when samples were sonicated; %recovery for pre-sonicated samples were as low as 67.3% whereas post-sonicated samples retained most of ASA concentrations at all QC levels. The results showed that sonication induced more efficient extraction of ASA from VAMS samples (Table 2).

Comparison between different sampling methods

To investigate possible differences between the two sampling methods, the ASA concentrations in the venous blood sample and the sample collected by VAMS were compared. Human blood was collected in ethylene diamine tetraacetic acid (EDTA) tubes and the aliquots were stored at -70°C and used for further analysis. As compared to nominal

Table 1. Accuracy and precision of the analytical method for ASA in whole blood, with stabilizer added

Concentration (ng/mL)	Mean (ng/mL)	Precision (CV%)	Accuracy (%)
Intra-day			
40	38.9	5.4	97.3
80	78.5	2.6	98.1
100	109.6	5.0	109.6
400	383.8	1.1	96.0
800	733.8	1.5	91.7
1,000	1,074.0	0.9	107.4
Inter-day			
40	40.7	6.9	101.8
80	78.1	3.5	97.6
100	105.1	5.0	105.1
400	381.8	3.6	95.5
800	747.6	3.1	93.5
1,000	1,068.0	1.4	106.8

ASA, acetylsalicylic acid; CV%, coefficient of variation.

Table 2. Comparison of ASA extraction efficiency between pre-sonicated and post-sonicated VAMS samples

Concentration (ng/mL)	Mean (ng/mL)	Recovery (%)
Pre-sonicated		
40	28.1	70.3
400	269.0	67.3
800	628.0	78.5
Post-sonicated		
40	40.0	100.0
400	396.0	99.0
800	791.0	98.9

ASA, acetylsalicylic acid; VAMS, volumetric absorptive microsampling.

concentrations (40, 400, and 800 ng/mL), the precision (CV%) values for venous blood samples were 2.7, 2.4, and 12.2% and values for VAMS samples were 1.8%, 3.5%, and 14.6%, respectively. In addition, the accuracy (%bias) values for venous blood samples were 111.0%, 100.4%, and 95.5% and the values for VAMS samples were 106.3%, 97.5%, and 97.3%, respectively; all the accuracy values were within the range of 85–115% (**Table 3**). Therefore, the results of ASA concentration analysis did not differ between sampling methods.

Stability of ASA on VAMS

The recovery of ASA from VAMS samples without additional stabilizer (mean, 31.3%) was approximately one third of the recovery from VAMS samples with stabilizer (mean, 100.3%) (**Table 4**). Hence, without additional stabilizer, the concentration of ASA in samples collected by VAMS would be lower than expected.

After applying the drying methods to prevent hydrolysis of ASA, the %recovery values observed for the dried VAMS samples in desiccator with calcium chloride (A), hypoxia chamber with nitrogen (B), desiccator with vacuum (C) and household vacuum sealer (D), were in the range of 92.0–99.6% (**Table 5, Fig. 3**). In addition, long-term stability was tested by keeping the samples for one month and then analyzing the concentration of ASA, and the %recovery values were in the range of 87.7–101.0% (**Table 6**). Even when the samples were sealed with household vacuum sealer and were stored for a longer period of time at 4°C and –20°C, the ASA recovery did not fall below 85%.

DISCUSSION

The differences between ASA concentrations were very small when VAMS samples dried under 4 different conditions were compared to the control. This indicated that the step of drying VAMS without control of humidity or temperature was the main cause of ASA instability, most likely by hydrolysis. Under all of the 4 different drying conditions, ASA

Table 3. Comparison of ASA concentration collected by conventional and VAMS sampling methods

Concentration (ng/mL)	Mean (ng/mL)	Precision (CV%)	Accuracy (%bias)
Conventional venous blood sampling			
40	44.4	2.7	111.0
400	401.6	2.4	100.4
800	764.3	12.2	95.5
VAMS sampling			
40	42.5	1.8	106.3
400	390.0	3.5	97.5
800	778.0	14.6	97.3

ASA, acetylsalicylic acid; VAMS, volumetric absorptive microsampling; CV%, coefficient of variation.

Table 4. Stability of ASA collected by VAMS with or without stabilizer added

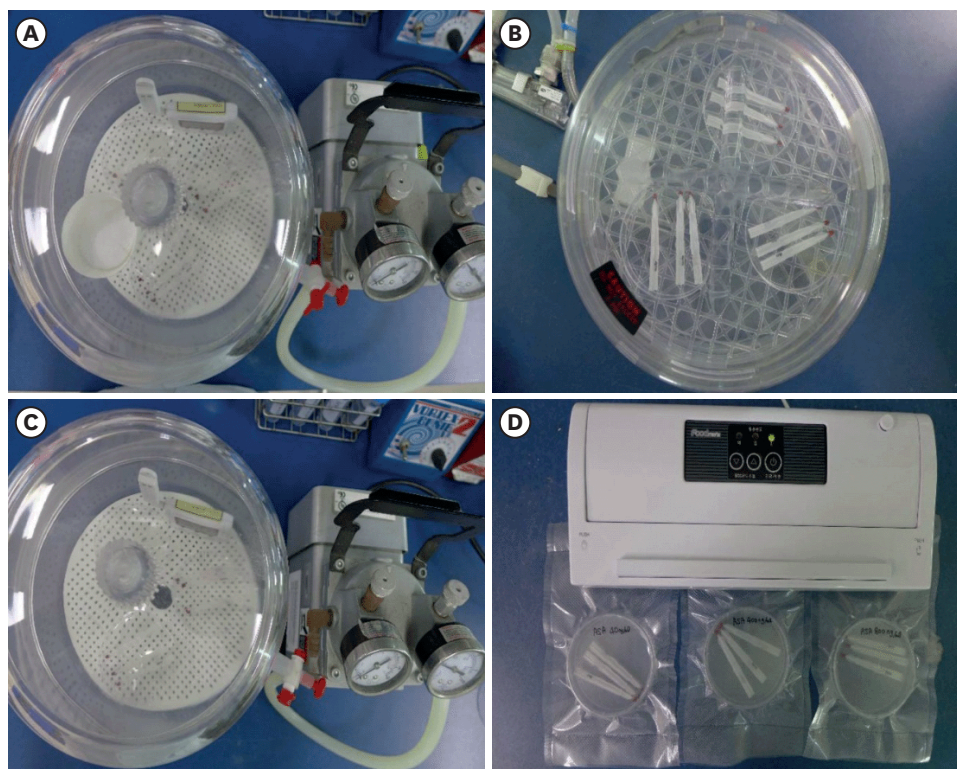
Concentration (ng/mL)	Mean (ng/mL)	Precision (CV%)	Accuracy (%)
With stabilizer added			
40	42.5	1.8	106.3
400	390.0	3.5	97.5
800	778.0	14.6	97.3
Without stabilizer added			
40	14.4	0.1	36.0
400	125.0	2.1	31.3
800	212.3	0.4	26.5

ASA, acetylsalicylic acid; VAMS, volumetric absorptive microsampling; CV%, coefficient of variation.

Table 5. Quantitative analysis of ASA collected by conventional and VAMS sampling method under various drying conditions for 1 hour

Drying conditions	Concentration (ng/mL)	Mean (ng/mL)	Precision (CV%)	Recovery (%)
Conventional venous blood sampling				
Room temperature	40	44.4	2.7	111.0
	400	401.6	2.4	100.4
	800	764.3	12.2	95.5
VAMS sampling				
Room temperature	40	13.8	0.7	34.5
	400	131.0	9.5	32.8
	800	210.0	2.5	26.3
Desiccator with calcium chloride	40	36.8	1.4	92.0
	400	398.3	15.0	99.6
	800	797.3	8.2	99.7
Hypoxia chamber with nitrogen	40	39.1	0.5	97.8
	400	382.3	1.1	95.6
	800	747.0	3.0	93.4
Desiccator with vacuum	40	38.4	0.3	96.0
	400	384.0	7.9	96.0
	800	751.0	11.5	93.9
Household vacuum sealer	40	38.2	0.4	95.5
	400	379.6	7.0	94.9
	800	746.6	8.0	93.3

ASA, acetylsalicylic acid; VAMS, volumetric absorptive microsampling; CV%, coefficient of variation.

**Figure 3.** Photographs illustrating the drying conditions proposed for VAMS samples to obtain dehumidification conditions. (A) DCC, (B) HCN, (C) DVC, and (D) HVS.

VAMS, volumetric absorptive microsampling; DCC, desiccator with calcium chloride; HCN, hypoxia chamber with nitrogen; DVC, desiccator with vacuum; HVS, household vacuum sealer.

Table 6. Long-term stability of ASA collected by conventional and VAMS sampling methods under various drying conditions after long-term storage of 1 month

Drying conditions	Concentration (ng/mL)	Mean (ng/mL)	Precision (CV%)	Recovery (%)
Conventional venous blood sampling				
Room temperature	40	44.4	2.7	111.0
	400	401.6	2.4	100.4
	800	764.3	12.2	95.5
VAMS sampling				
Desiccator with calcium chloride	40	39.8	2.1	99.5
	400	399.6	6.1	99.9
	800	779.0	6.1	97.4
Hypoxia chamber with nitrogen	40	36.7	1.4	91.8
	400	404.3	2.8	101.1
	800	798.6	4.9	99.8
Desiccator with vacuum	40	37.7	1.4	94.3
	400	400.3	1.6	100.0
	800	780.0	6.1	97.5
Household vacuum seale	40	36.2	0.8	90.5
	400	384.3	5.9	96.1
	800	752.0	11.0	94.0
Household vacuum sealer (4°C)	40	35.1	0.9	87.8
	400	391.0	2.9	97.8
	800	750.6	5.1	93.8
Household vacuum sealer (-20°C)	40	35.1	0.9	87.8
	400	400.0	8.0	100.0
	800	751.6	3.0	94.0

ASA, acetylsalicylic acid; VAMS, volumetric absorptive microsampling; CV%, coefficient of variation.

remained stable in each proposed environment across all concentrations of 40, 400 and 800 ng/mL. ASA stability was a parameter affected by humidity [24]; the drying condition that yielded highest recovery was A, desiccator with humidity level lowered to 11%, followed by B, hypoxia chamber filled with 99% nitrogen, and C, pressurized desiccator and the last, D, household vacuum sealer.

Stability is a critical parameter associated with sample integrity and data consistency in bioanalysis. ASA is associated with negative connotations such as “unstable” due to its degradation-prone characteristics, having an ester functional group that is readily hydrolyzed when in contact with moisture. Ester group is not the only functional group that is prone to degradation; it has been observed that compounds containing amide, lactam, lactone and chloride exhibit similar chemical characteristics [24]. There are many environmental factors that are responsible in degradation of analytes, such as temperature, humidity and sunlight. However, humidity that causes active pharmaceutical ingredients to undergo hydrolysis is by far the most common factor that causes degradation of drugs [25]. The results of our study that experimented different drying conditions by attempting to keep the samples independent of surrounding air were in good agreement with the findings of the aforementioned publications that moisture is a critical factor in drug stability maintenance. It is also predicted that the results of our research could be extended to analysis of other drugs containing other functional groups of similar chemical properties. To name a few, acetaminophen, chloramphenicol, oxazepam, chloridazepoxide, penicillin and warfarin would be potential candidates given their susceptibility to hydrolysis.

In this study, we have used hydrochlorothiazide as the IS, because it is a compound structurally similar to ASA; by using hydrochlorothiazide as the IS, a clean peak could be obtained without interference, with a fast detection time with proper retention time similar

to those of ASA [26]. In addition, the analysis of ASA with hydrochlorothiazide as the IS showed that it was possible to detect low concentrations of ASA by optimizing the solvent buffer and column, and the fast detection time had the advantage of accelerating the time required for the analysis.

Growing body of academic evidence suggests that patient involvement and satisfaction could render better clinical research outcome and various attempts by pharmaceutical companies have been made to emphasize patient-centricity in clinical research. Patient-centricity includes initiatives such as innovative partnerships, technology advancements, simplified protocol design and easier participation for study volunteers [27-29], all of which are required to give patients power and knowledge about clinical trial participation. Despite gradual progress, recent findings suggest that the current framework is still lagging behind; recruitment and patient adherence are critical to the success of clinical trials, but sites are failing to recruit and retain patients [30]. The need for measures to further bolster patient-centricity is greater nowadays, and VAMS could aid reinforcing the concept. However, given novelty of the device, its application for different drugs has not been confirmed.

VAMS will be particularly useful in clinical scenarios where frequent hospital visits are required for blood concentration monitoring as in cases of diabetes or therapeutic drug monitoring. Although further research is needed that investigates the effect of environmental factors that could affect the analytes during the process of drying, storage and transport, our study is a step forward in the investigation of such factors affecting the stability of ASA. ASA is hydrolyzed by esterase and is readily converted to salicylic acid when in contact with moisture; typically, potassium fluoride and sodium fluoride are added as stabilizers to prevent rapid degradation in clinical practice. In an attempt to expand the scope of VAMS utilization to ASA, we devised a method of preventing hydrolysis in the experimental setting to ensure stability of the drug analyte without adding stabilizer, by using desiccants and vacuums to control humidity. The current study is meaningful because we have found out that blood samples collected by VAMS could be stored in stable conditions using just a simple household vacuum sealer, without adding stabilizer, for up to 1 month; this means that patients could get their blood ASA levels tested without paying a visit to hospitals.

To our knowledge, this is the first paper to investigate a method of stabilizing drugs susceptible to degradation during the storage step *in vitro*. Also by presenting a storage and transport method that does not require freezing, we have also shown a possibility of blood sampling that can be done at home, with reliability and convenience. Despite the limitation of the method that it was tested under laboratory conditions, we have successfully secured stability of the drug without adding stabilizing reagent to the samples.

In conclusion, our study has demonstrated a prospect of VAMS utilization in the sampling of ASA, well known for hydrolytic degradation stemming from chemical instability. For the quantitation of ASA, an LC-MS/MS analysis method, with simple protein precipitation and adequate reproducibility and reliability, was developed. It was fully validated in terms of sensitivity, specificity, linearity, accuracy, precision and stability. Adequate level of humidity control was critical to ensure sample stability of ASA, and various drying and storage conditions were tested to report the %recovery kept above 85%. Although the experiment was conducted under laboratory conditions, this study successfully addressed sample stability issues that could occur during drying, storage and transport of VAMS sample. Because blood samples of ASA collected by VAMS could be stored in stable conditions using just a

simple household vacuum sealer, without adding stabilizer, for up to 1 month, the results of our study could enable convenient blood sampling at home using VAMS; this could lead to minimized hospital visits and patient-centric clinical trials to be conducted, and the results could also be extended to other compounds with functional groups susceptible to degradation.

REFERENCES

1. Warner TD, Mitchell JA. Cyclooxygenase-3 (COX-3): filling in the gaps toward a COX continuum? *Proc Natl Acad Sci U S A* 2002;99:13371-13373.
[PUBMED](#) | [CROSSREF](#)
2. Ittaman SV, VanWormer JJ, Rezkalla SH. The role of aspirin in the prevention of cardiovascular disease. *Clin Med Res* 2014;12:147-154.
[PUBMED](#) | [CROSSREF](#)
3. University of Rochester Medical Center. Salicylate (blood) [Internet]. https://www.urmc.rochester.edu/encyclopedia/content.aspx?contenttypeid=167&contentid=salicylate_blood. Accessed March 19, 2020.
4. Arif H, Aggarwal S. Salicylic acid (aspirin). *StatPearls* [Internet]. <https://www.ncbi.nlm.nih.gov/books/NBK519032/>. Updated July 15, 2021. Accessed January 10, 2022.
5. Pachman LM, Olufs R, Procknal JA, Levy G. Pharmacokinetic monitoring of salicylate therapy in children with juvenile rheumatoid arthritis. *Arthritis Rheum* 1979;22:826-831.
[PUBMED](#) | [CROSSREF](#)
6. Koren G, MacLeod SM. Difficulty in achieving therapeutic serum concentrations of salicylate in Kawasaki disease. *J Pediatr* 1984;105:991-995.
[PUBMED](#) | [CROSSREF](#)
7. Denniff P, Spooner N. Volumetric absorptive microsampling: a dried sample collection technique for quantitative bioanalysis. *Anal Chem* 2014;86:8489-8495.
[PUBMED](#) | [CROSSREF](#)
8. Leuthold LA, Heudi O, Déglon J, Raccuglia M, Augsburg M, Picard F, et al. New microfluidic-based sampling procedure for overcoming the hematocrit problem associated with dried blood spot analysis. *Anal Chem* 2015;87:2068-2071.
[PUBMED](#) | [CROSSREF](#)
9. Kok MG, Fillet M. Volumetric absorptive microsampling: current advances and applications. *J Pharm Biomed Anal* 2018;147:288-296.
[PUBMED](#) | [CROSSREF](#)
10. Patel P, Mulla H, Tanna S, Pandya H. Facilitating pharmacokinetic studies in children: a new use of dried blood spots. *Arch Dis Child* 2010;95:484-487.
[PUBMED](#) | [CROSSREF](#)
11. Kip AE, Kiers KC, Rosing H, Schellens JH, Beijnen JH, Dorlo TP. Volumetric absorptive microsampling (VAMS) as an alternative to conventional dried blood spots in the quantification of miltefosine in dried blood samples. *J Pharm Biomed Anal* 2017;135:160-166.
[PUBMED](#) | [CROSSREF](#)
12. Barfield M, Spooner N, Lad R, Parry S, Fowles S. Application of dried blood spots combined with HPLC-MS/MS for the quantification of acetaminophen in toxicokinetic studies. *J Chromatogr B Analyt Technol Biomed Life Sci* 2008;870:32-37.
[PUBMED](#) | [CROSSREF](#)
13. Parker SL, Guerra Valero YC, Lipman J, Roberts JA, Wallis SC. Effect of time on recovery of plasma microsamples for the quantitative determination of vancomycin. *Bioanalysis* 2016;8:2235-2242.
[PUBMED](#) | [CROSSREF](#)
14. Vane JR, Botting RM. The mechanism of action of aspirin. *Thromb Res* 2003;110:255-258.
[PUBMED](#) | [CROSSREF](#)
15. Gu N, Kim BH, Chung YJ, Lim KS, Seo HB, Yim DS, et al. Influence of simvastatin on pharmacokinetics/pharmacodynamics of aspirin after oral co-administration in healthy volunteers. *J Kor Soc Clin Pharm Ther* 2011;19:73-83.
[CROSSREF](#)
16. Feldman M, Shewmake K, Cryer B. Time course inhibition of gastric and platelet COX activity by acetylsalicylic acid in humans. *Am J Physiol Gastrointest Liver Physiol* 2000;279:G1113-G1120.
[PUBMED](#) | [CROSSREF](#)

17. Kim Y, Jeon JY, Han SH, Ha N, Jang K, Kim MG. Quantitative analysis of acetylsalicylic acid in human blood using volumetric absorptive microsampling. *Transl Clin Pharmacol* 2018;26:32-38.
[PUBMED](#) | [CROSSREF](#)
18. Parker SL, Roberts JA, Lipman J, Wallis SC. Quantitative bioanalytical validation of fosfomycin in human whole blood with volumetric absorptive microsampling. *Bioanalysis* 2015;7:2585-2595.
[PUBMED](#) | [CROSSREF](#)
19. Marahatta A, Megaraj V, McGann PT, Ware RE, Setchell KD. Stable-isotope dilution HPLC-electrospray ionization tandem mass spectrometry method for quantifying hydroxyurea in dried blood samples. *Clin Chem* 2016;62:1593-1601.
[PUBMED](#) | [CROSSREF](#)
20. Xie I, Xu Y, Anderson M, Wang M, Xue L, Breidinger S, et al. Extractability-mediated stability bias and hematocrit impact: High extraction recovery is critical to feasibility of volumetric adsorptive microsampling (VAMS) in regulated bioanalysis. *J Pharm Biomed Anal* 2018;156:58-66.
[PUBMED](#) | [CROSSREF](#)
21. Korean Ministry of Food and Drug Safety. Guideline on bioanalytical method validation. Cheongju: Korean Ministry of Food and Drug Safety; 2013.
22. Kelly CA. Determination of the decomposition of aspirin. *J Pharm Sci* 1970;59:1053-1079.
[PUBMED](#) | [CROSSREF](#)
23. Suslick KS, Fang MM, Hyeon T, Mdleleni MM. Applications of sonochemistry to materials synthesis. In: Crum LA, Mason TJ, Reisse JL, Suslick KS (eds). *Sonochemistry and sonoluminescence*. Dordrecht: Springer; 1999, 291-320.
24. Roy S, Siddique S, Majumder S, Abdul MI, Rahman SA, Lateef D, et al. A systemic approach on understanding the role of moisture in pharmaceutical product degradation and its prevention: challenges and perspectives. *Biomed Res (Aligarh)* 2018;29:3336-3343.
[CROSSREF](#)
25. Waterman KC, Adami RC, Alsante KM, Antipas AS, Arenson DR, Carrier R, et al. Hydrolysis in pharmaceutical formulations. *Pharm Dev Technol* 2002;7:113-146.
[PUBMED](#) | [CROSSREF](#)
26. Halder D, Dan S, Biswas E, Sarkar P, Halder U, Pal T. A rapid LC-ESI-MS/MS method for the quantitation of salicylic acid, an active metabolite of acetylsalicylic acid: application to in vivo pharmacokinetic and bioequivalence study in Indian healthy male volunteers. *Appl Clin Res Clin Trials Regul Aff* 2015;2:90-102.
[CROSSREF](#)
27. Yeoman G, Furlong P, Seres M, Binder H, Chung H, Garzya V, et al. Defining patient centricity with patients for patients and caregivers: a collaborative endeavour. *BMJ Innov* 2017;3:76-83.
[PUBMED](#) | [CROSSREF](#)
28. Robbins DA, Curro FA, Fox CH. Defining patient-centricity: opportunities, challenges, and implications for clinical care and research. *Ther Innov Regul Sci* 2013;47:349-355.
[PUBMED](#) | [CROSSREF](#)
29. Lamberti MJ, Awatin J. Mapping the landscape of patient-centric activities within clinical research. *Clin Ther* 2017;39:2196-2202.
[PUBMED](#) | [CROSSREF](#)
30. Chhatre S, Jefferson A, Cook R, Meeker CR, Kim JH, Hartz KM, et al. Patient-centered recruitment and retention for a randomized controlled study. *Trials* 2018;19:205.
[PUBMED](#) | [CROSSREF](#)