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Analytical performance evaluation of hemoglobin A1c on an ARKRAY HA-8160 analyzer with newly-developed mobile phase buffer

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ABSTRACT

Background: Most glycated hemoglobin A1c (HbA1c) analytical reagents used were obtained from the analyzer's manufacturer. However, clinical laboratories need more choices for HbA1c analytical reagents to overcome the limitations of dedicated reagents for special analyzers. We developed new mobile phase buffers as HbA1c diagnostic reagents and evaluated their analytical performance for the HbA1c assay.

Methods: Different mobile phase buffers used as HbA1c diagnostic reagents were prepared using different concentrations of sodium salts. According to the Clinical and Laboratory Standards Institute (CLSI) recommendation guidelines, the analytical performances of the newly developed mobile phase buffers were evaluated on an ARKRAY HA-8160 Analyzer. Both quality controls and clinical blood samples were used in these experiments. To assess the quality of the newly developed mobile phase buffers, precision, accuracy, linearity, carryover, interference, bias, correlation with commercial reagents, and stability were analyzed.

Results: The *CVs* of intra-assay precision and interassay precision of quality control and clinical. There were fewer than 1.00 % blood sample assays using the newly developed mobile phase buffer. The *RDs* of accuracy were less than 1.00 %. Linearity: $R^2 = 0.9998$ in the concentration range of 4.40%–17.30 %. Carryover: 0.00 %. Reagent comparison revealed that the Pearson regression equation was $Y = 0.9884x+0.05692$ ($R^2 = 0.9977$), and the Bland-Altman mean difference was −0.02650 % (CI: −0.2121 %–0.1591 %) between the two analytical reagents. Stability was also acceptable within 12 months. This mobile phase buffer showed good anti-interference ability.

Conclusion: The newly developed mobile phase buffers demonstrated good analytical performance and were suitable for clinical HbA1c assays on an ARKRAY HA-8160 Analyzer.

1. Introduction

HbA1c is widely used to diagnose and monitor glycemic control in people with diabetes mellitus [1]. Given the importance of

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HbA1c as a guide for glycemic control and diagnosis, precise HbA1c measurement is essential for patients with diabetes. Various methods are used for its determination. To date, many methods, such as enzymatic assays [2], capillary electrophoresis [3,4], boronate affinity chromatography [5], ion-exchange high-performance liquid chromatography (ion-exchange HPLC) [6] and immunoassays [7], have been developed and updated. Ion-exchange HPLC assays based on the charge difference principle are among the most commonly used methods for measuring HbA1c in laboratories [8,9]. This analytical system usually includes an automatic glycohemoglobin analyzer, eluents, hemolysis & wash solution, and a chromatography column [10]. The glycated hemoglobin analyzer provides chromatographic conditions such as flow rate, temperature, and sample loading volume/concentration chromatographic conditions, and the chromatography column, as a hemoglobin adsorption and dissociation carrier, is the core of the components, which usually cannot be modified after leaving the factory.

In addition to the analyzer and column, the chromatographic conditions that can be changed include mobile phase buffers with the right selectivity, which are critical for achieving the desired separation [11,12]. The precise analysis still requires a specific elution buffer and hemolysis & wash solution [13]. Hemolysis & wash solution is a cell lysis buffer that disrupts erythrocytes to release hemoglobin and clean equipment pipes. Eluents can provide a ladder of eluent conditions for the HbA1c assay. The mobile phase is an important factor for the HbA1c assay. However, the use of mobile phase buffers as glycated hemoglobin reagents is confidential to the analyzer's manufacturer. To date, most studies have focused on comparing the performance of different automatic glycohemoglobin analyzers or hemoglobin testing systems [14–17]. Few studies have focused on the preparation of mobile phase buffers [18] or the analytical performance evaluation of different mobile phase buffers [10]. The use of mobile phase buffers, which are common consumables, needs to be frequently changed. Therefore, clinical laboratories need more options from different manufacturers to reduce the cost of ownership.

In this study, according to the function of each mobile phase buffer, the formula of the mobile phase buffer was optimized. Various sodium salts, preservatives, nonionic surfactants, and pH buffer systems were used to prepare different mobile phase buffers. All chemical reagents of each mobile phase buffer were prepared with deionized water and then vortexed. Finally, all the mobile phase buffers were filtered through 0.22 μm filters to avoid chromatography column blockage. Moreover, we assessed the comprehensive analytical performance (i.e., precision, linearity, accuracy, carryover, long-term stability, and anti-interference ability) of the mobile phase buffer on the ARKRAY HA-8160 Analyzer. We developed new mobile phase buffers that were compatible with the related analyzer system and produced reliable glycohemoglobin analysis results.

2. Materials and methods

2.1. Reagents and samples

The following chemical reagents met with high-performance liquid chromatography purity were used in this study: sodium azide (NaN_3), sodium perchlorate monohydrate ($NaClO_4{\cdot}H_2O$), disodium succinate hexahydrate ($C_4H_4Na_2O_4{\cdot}6H_2O$), succinic acid ($C_4H_6O_4$), sodium phosphate dibasic dodecahydrate ($Na_2HPO_4{\cdot}12H_2O$), sodium dihydrogen phosphate dehydrate ($NaH_2PO_4{\cdot}2H_2O$), Triton™ X-100 and Tris base were purchased from Aladdin (Shanghai Aladdin Biochemical Technology Co., Ltd., Shanghai, China). Deionized water and quality control solution were obtained from Lirimax (Lirimax (Tianjin) Medical Technology Co., Ltd., Tianjin, China). The eluent 61A, eluent 61B, eluent 61C, calibrator set, and chromatography column, and hemolysis & wash solution were obtained from ARKRAY (ARKRAY, Inc., Kyoto, Japan).

Human glycated hemoglobin reference materials (Cat No. GBW09181 and GBW09183) were obtained from the Clinical Laboratory Center of the Ministry of Health, Beijing Hospital in China. The leftover blood samples of the healthy volunteer volunteers were collected from the Department of Clinical Laboratory, Air Force Medical Center, and Air Force Medical University.

2.2. Instrument

The whole evaluation was conducted on an HA-8160 automatic glycohemoglobin analyzer (ARKRAY, Inc., Kyoto, Japan). The standard mode was utilized for HbA1c measurement. The analyzer was calibrated at the beginning of the measurement according to the manufacturer's instructions.

Table 1
The formula of mobile phase buffer.

Mobile phase	Composition	pH value
Eluent I	3.5141 g/L $NaClO_4{\cdot}H_2O$, 6.1669 g/L $C_4H_4Na_2O_4{\cdot}6H_2O$, 0.9475 g/L $Na_2HPO_4{\cdot}12H_2O$, 8.2071g/L $NaH_2PO_4{\cdot}2H_2O$, 0.2692 g/L NaN_3, 0.8371 g/L $C_4H_6O_4$, 1.2641 g/L tris base and 1.0L deionized water	5.15–5.55
Eluent II	9.7389 g/L $NaClO_4{\cdot}H_2O$, 0.8649 g/L $C_4H_4Na_2O_4{\cdot}6H_2O$, 31.5805 g/L $Na_2HPO_4{\cdot}12H_2O$, 2.2071g/L $NaH_2PO_4{\cdot}2H_2O$, 0.3499 g/L NaN_3, 0.7121 g/L tris base and 1.0L deionized water	7.85–8.25
Eluent III	2.2731 g/L $NaClO_4{\cdot}H_2O$, 2.1207 g/L $C_4H_4Na_2O_4{\cdot}6H_2O$, 3.2068g/L $Na_2HPO_4{\cdot}12H_2O$, 6.4228 g/L $NaH_2PO_4{\cdot}2H_2O$, 0.3112 g/L NaN_3, 0.8002 g/L $C_4H_6O_4$, 2.021 g/L tris base and 1.0L deionized water	5.15–5.55
Hemolysis & wash solution	1.0 mL/L Triton™ X-100, 0.31 g/L NaN_3 and 1.0L deionized water	7.0–7.2

2.3. Preparation of the mobile phase buffer

Based on the principle of ion-exchange HPLC, we chose sodium ions as the main ions for cation exchange to perform the HbA1c assay. Disodium hydrogen phosphate dodecahydrate and sodium dihydrogen phosphate anhydrous were used to construct stable pH buffers. Succinic acid was used to calibrate the pH values. Sodium azide is a preservative that also provides sodium ions. Sodium perchlorate monohydrate is the primary sodium ion provider and serves to clean the column. Triton™ X-100 was used as a nonionic surfactant to clean the column and all lines. In addition, a Tris base is used to keep the buffer stable at ambient temperatures, which can change its pH. According to the elution gradient and hemolysis requirements of the glycated hemoglobin analyzer, we prepared different ionic concentrations of eluents and hemolysis & wash solutions. The formulas of the eluents (Eluent I, Eluent II, and Eluent III) and the hemolysis & wash solutions are shown in Table 1. The eluents and hemolysis & wash solution were prepared in a clean factory workshop (Class: 10,000), vortexed for 60 min, and filtered through 0.22 μm PES filters. All eluents and hemolysis & wash solutions were stored at 10°C–30 °C.

2.4. Evaluation protocol

2.4.1. Precision study

Intra- and interassay precision were evaluated following the CLSI document EP5-A2 [19]. There were two sets of testing samples: quality controls and blood specimens. The quality controls included low values (R_1, HbA1c: 5.12 % [32.00 mmol/mol]) and high values (R_2, HbA1c: 10.20 % [82.00 mmol/mol]). The clinical blood specimens included low-value (L_1-L_3, HbA1c: 4.50%–5.30 % [25.68–34.43 mmol/mol]), medium-value (M_1-M_3, HbA1c: 7.00%–8.50 % [53.00–69.00 mmol/mol]) and high-value (H_1–H_3, HbA1c: 9.40%–16.80 % [79.25–160.14 mmol/mol]) samples. All the samples were aliquoted and stored at −70 °C to avoid freezing/thawing cycles.

For intra-assay precision evaluation, the same lots of eluents and hemolysis & wash solutions were utilized for 20 tests of quality control and clinical blood specimens on the calibrated analyzer.

For interassay precision evaluation, the clinical blood specimens and quality control were measured 20 times with 3 lots of eluents and hemolysis & wash solution on a calibrated analyzer. The mean, coefficient of variation (*CV*), and standard deviation (*SD*) were calculated.

2.4.2. Accuracy study

Accuracy was evaluated following the CLSI Guidelines EP10-A3 [20]. The reference materials of the glycated hemoglobins (No. GBW09181a, GBW09182a, and GBW09183a) were measured 5 times on a calibrated analyzer. The mean, SD, and relative deviation (*RD*) were calculated.

2.4.3. Linearity study

The linearity study was performed in accordance with CLSI Guideline EP6-A [21]. One low-value blood specimen (HbA1c: 4.40 % [25.00 mmol/mol]) and one high-value blood specimen (HbA1c: 17.30 % [166.00 mmol/mol]) were included. Then, the samples were diluted 80-fold, and the samples were mixed at ratios of 2:0, 1.6:0.4, 1.2:0.8, 1.0:1.0, 0.8:1.2, 0.4:1.6, and 0:2 for a total of 7 concentrations with low and high HbA1c blood pools. Then, seven mixed samples were obtained and measured 3 times on the calibrated analyzer. Pearson regression was used to compare target and measured values.

2.4.4. Carryover study

Carryover was evaluated in accordance with CLSI Guideline EP10-A3 [20]. Briefly, one low-value Hb1Ac (HbA1c: 4.50%–5.30 % [25.68–34.43 mmol/mol]) and one high-value Hb1Ac (Hb1Ac 10.60%–16.80 % [92.37–160.14 mmol/mol]) blood sample were measured as follows. The repeated injections of low- and high-value blood samples were L_1-L_5 and H_1–H_4, which were subsequently measured in the order of L_1-L_2-L_3-L_4-H_1-H_2-H_3-H_4-L_5. The data were analyzed for carryover determination.

2.4.5. Bias and correlation

The comparison and bias estimation of different mobile phase buffers were performed according to CLSI guidelines EP9-A3 [22]. A total of 120 clinical patient samples with HbA1c values over a clinically relevant range (4.50 % [25.68 mmol/mol] to 16.80 % [160.14 mmol/mol]) were used in this study. The measurements were conducted using newly developed mobile phase buffers and ARKRAY reagents. The analyzer was initially calibrated using their respective calibrators. Pairs of HbA1c measurement values were then compared with Pearson regression.

2.4.6. Stability study

The stability test was performed in accordance with CLSI Guideline EP25-A [23]. For long-term stability of the mobile phase buffer (stored at 10°C–30 °C), the performance of the mobile phase buffer was evaluated by quality control measurements at the 3rd, 6th, 9th, and 12th months, as described above.

2.4.7. Interference assessment

The interference assessment was performed in accordance with CLSI Guideline EP07-A2 [24]. The following potential endogenous and exogenous interfering substances were selected for the HbA1c assay: triglycerides (20.0 mmol/L), hemoglobin (2.0 g/L), uric acid

(20.0 mmol/L), ethanol (86.8 mmol/L) and vitamin C (114.0 μmol/L). Potential interference was evaluated in two-level samples at high and low levels of HbA1c. Samples with or without specific concentrations of interferents were prepared and analyzed in three replicates.

2.4.8. Statistical analysis

Statistics were performed using GraphPad Prism software, version 5.0 (GraphPad Software Inc., La Jolla, CA). The data are presented as the means, *SDs*, *CVs*, *RDs*, and correlation coefficients (R^2).

3. Results

3.1. Precision

Both the intra-assay and interassay precision analyses are summarized in Table 2 and Table 3, respectively. Both quality control of HbA1c and clinical blood samples were used. All of the *CVs* were lower than 1.00 %. A CV less than 2.00 % met the National Health Commission of the People's Republic of China's health industry standard: Measurement of hemoglobin A1c [10].

3.2. Accuracy

The accuracy was evaluated by measuring 3 levels of reference materials. The bias results are shown in Table 4. All of the *RDs* were less than 1.00 %, which meets the National Health Commission of the People's Republic of China-issued health industry standard: Measurement of hemoglobin A1c [10].

3.3. Linearity

As shown in Fig. 1, the HbA1c measurements showed good linearity from 4.40 % to 17.30 % (25.00–166.00 mmol/mol) across the assay range. Linear regression showed that the measured values matched the target values well ($R^2 = 0.9998$), indicating good linearity.

3.4. Carryover

The carryover between high- and low-level HbA1c samples measured was 0.00 %, less than 1.00 %, meeting the allowable acceptance criterion of carryover.

3.5. Bias and correlation

HbA1c measurement values were compared with data from both ARKRAY and newly developed mobile phase buffer assays. As shown in Fig. 2, the linear regression equation was $Y = 0.9884x+0.05692$, and the Pearson correlation coefficient was 0.9977. The 95 % confidence intervals were between 0.9799 and 0.9970 for the slope, the Y-intercept values ranged from −0.007028 to 0.1209, and the X-intercept values ranged from −0.1233 to 0.007051. These results indicated slight constant and proportional errors.

The Bland-Altman plot showed a mean difference from 0.2121 to 0.1591 with 0.083 % outliers (differences outside the *mean ± 1.96 SD range*) in Fig. 3. The Bland-Altman mean difference was −0.02650 % (CI: −0.2121 %–0.1591 %) between the two analytical reagents. These two comparison studies for linearity indicated no significant deviation (*P* value > 0.05).

3.6. Stability

The mobile phase buffers were stored at 10°C–30 °C. The stability of the mobile phase buffer was evaluated on the 3rd, 6th, 9th, and 12th month, respectively. The accuracy, precision, and linearity of the mobile phase buffer are shown in Table 5, Table 6, and Fig. 4, respectively. All the results are acceptable according to the common criteria.

3.7. Interference

As shown in Table 7, the relative biases of five potential interfering substances, namely, triglycerides (up to 20.0 mmol/L),

Table 2
Precision as evaluated by analysis of quality control at two levels.

Quality Control	Target Value	Intra-assay precision		Inter-assay precision	
		Mean ± SD (%)	*CV* (%)	Mean ± SD (%)	*CV* (%)
R1	5.12 % (32.00 mmol/mol)	5.11 ± 0.03	0.60	5.13 ± 0.05	0.91
R2	10.20 %(82.00 mmol/mol)	10.21 ± 0.02	0.22	10.24 ± 0.05	0.49

Table 3
Precision as evaluated by analysis of clinical blood samples at three levels.

Sample	Intra-assay precision		Inter-assay precision	
	Mean ± SD (%)	CV (%)	Mean ± SD (%)	CV (%)
L1	5.04 ± 0.05	0.74	5.04 ± 0.03	1.00
L2	4.86 ± 0.05	0.45	4.85 ± 0.05	1.00
L3	5.22 ± 0.04	0.70	5.23 ± 0.05	0.88
M1	6.74 ± 0.05	0.73	6.75 ± 0.06	0.84
M2	7.10 ± 0.01	0.11	7.13 ± 0.05	0.67
M3	7.66 ± 0.05	0.66	7.64 ± 0.03	0.73
H1	10.71 ± 0.03	0.29	10.77 ± 0.06	0.59
H2	13.52 ± 0.04	0.30	13.59 ± 0.07	0.52
H3	16.82 ± 0.06	0.37	16.89 ± 0.05	0.28

Table 4
Accuracy study, differences between measured and target values.

Reference material sample	Target value		Measurement Value(NGSP unit)	
	NGSP unit (%)	*IFCC unit* (mmol/mol)	Mean ± SD (%)	RD (%)
GBW09181a	5.02 ± 0.25	31.36 ± 0.91	5.00 ± 0.00	−0.398
GBW09182a	6.86 ± 0.15	51.49 ± 1.14	6.84 ± 0.055	−0.214
GBW09183a	9.34 ± 0.21	78.60 ± 1.76	9.32 ± 0.045	−0.292

NGSP: National Glycohemoglobin Standardization Program.
IFCC: International Federation of Clinical Chemistry and Laboratory Medicine.

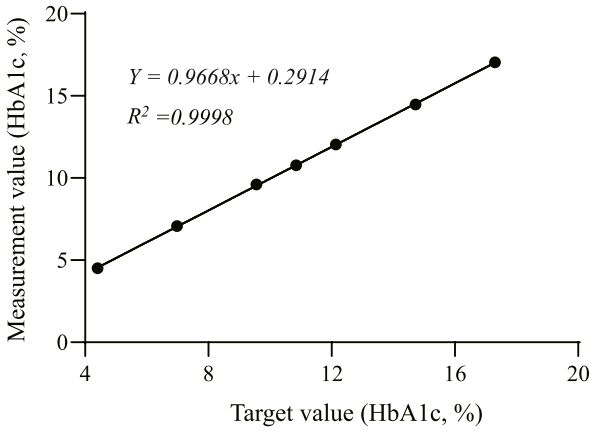

Fig. 1. The linearity was evaluated by linear regression analysis to compare correlation between measurement values and target values of HbA1c. Range: 4.40%–17.30 % (25.00mmol/mol-166.00 mmol/mol).

hemoglobin (up to 2.0 g/L), uric acid (up to 20.0 mmol/L), ethanol (up to 86.8 mmol/L) and vitamin C (up to 114.0 μmol/L), were less than 1.0 %, lower than 6.0 %, as reported by the National Glycohemoglobin Standardization Program [25], indicating that there was no significant analytical interference with the mobile phase buffers at the tested concentrations of these interfering substances.

4. Discussion

The HbA1c assay plays a crucial role in the monitoring and diagnosis of diabetes, and its clinical use must be supported by standardized results such as accuracy and equivalence among different measurement methods and clinical laboratories [26]. The ion exchange HPLC assay has been recommended by the National Glycohemoglobin Standardization Program [6]. The components of the HPLC system included a mobile phase buffer for hemolysis & wash solution, elution buffer, and a stationary phase chromatography column. Although there are a number of studies evaluating these analyzers or comparing different analyzers [27], few studies have evaluated the performance of mobile phase buffers or whether the diagnostic kit was suitable for certain analyzers. Although the analyzer plays an important role in the HPLC measurement system, the performance of the mobile phase buffer is also crucial for the reliability of the HbA1c assay [28].

Generally, the mobile phase buffers used as glycosylated hemoglobin reagents are supplied by the analyzer manufacturer. There are few alternative reagents from nonanalyzer manufacturers because the mobile phase contains a variety of factors, such as the ion

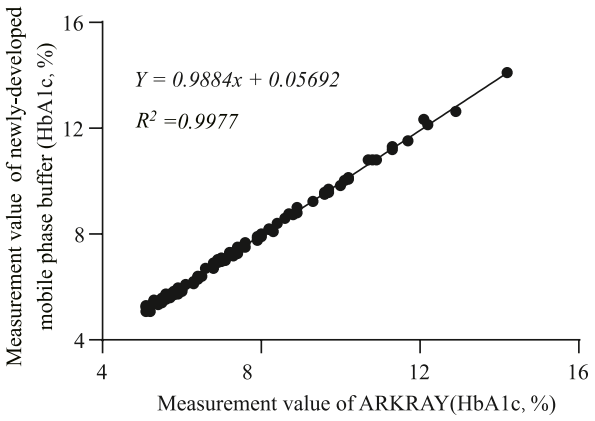

Fig. 2. Correlation of HbA1c value measured with reagents from ARKRAY and newly developed mobile phase buffer. $R^2 = 0.9977$.

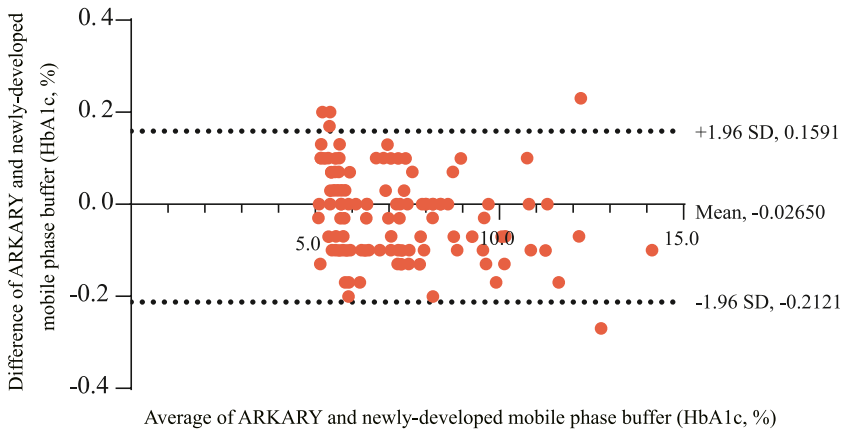

Fig. 3. Comparison between ARKRAY reagents vs newly developed mobile phase buffer, Bland-Altman plot, and mean difference is −0.02650 %.

Table 5
Stability study, accuracy analysis of newly developed mobile phase buffer.

Reference material	Target value	Month	Mean ± SD (%)	RD (%)
GBW09181a	5.02 ± 0.25 (%) (31.36 ± 0.91 mmol/mol)	3rd	5.00 ± 0.00	−0.398
		6th	4.96 ± 0.055	−1.200
		9th	4.99 ± 0.046	−0.597
		12th	5.04 ± 0.036	0.398
GBW09183a	9.34 ± 0.21 (%) (78.60 ± 1.76 mmol/mol)	3rd	9.36 ± 0.034	0.214
		6th	9.40 ± 0.044	0.642
		9th	9.30 ± 0.032	−0.428
		12th	9.27 ± 0.054	−0.749

concentration and pH, that influence the retention time of the column. The pH of mobile phase buffers is susceptible to ambient temperature factors. Therefore, this is a great challenge for the stability of mobile phase buffers. In this study, disodium hydrogen phosphate dodecahydrate and sodium dihydrogen phosphate anhydrous were used to construct a dynamically balanced buffer, and Tris base was also used to maintain a stable pH buffer system [29,30]. In addition, we used a constant pH range and optimized the ionic concentration of each eluent to establish the ionic formulation of each eluent. According to the functional requirements of each mobile phase buffer, the hemolysis & wash solution was prepared with deionized water, Triton™ X100 as a nonionic surfactant, and an ultratrace concentration of sodium azide as a preservative, a hypotonic buffer that can lyse erythrocytes and strongly cleave equipment pipes. Eluent I, Eluent II, and Eluent III were prepared with different concentrations of sodium azide, sodium perchlorate monohydrate, disodium succinate hexahydrate, succinic acid, sodium phosphate dibasic dodecahydrate, and sodium dihydrogen phosphate dehydrate, respectively. These eluents can provide a range of eluent conditions for the HbA1c assay. Sodium azide has preservative and stabilizing capabilities and provides sodium ions [31]. The different ratios of sodium phosphate dibasic dodecahydrate and sodium dihydrogen phosphate dehydrate can provide a stable pH buffer [32]. Sodium perchlorate monohydrate and disodium succinate

Table 6
Stability study, Precision analysis of newly developed mobile phase buffer.

Quality Control	Target value	Month	Intra-assay precision	
			Mean ± SD (%)	CV (%)
R1	5.12 % (32.00 mmol/mol)	3rd	5.11 ± 0.03	0.60
		6th	5.12 ± 0.03	0.34
		9th	5.13 ± 0.04	0.45
		12th	5.12 ± 0.03	0.39
R2	10.20 % (82.00 mmol/mol)	3rd	10.20 ± 0.03	0.46
		6th	10.22 ± 0.03	0.42
		9th	10.21 ± 0.05	0.54
		12th	10.23 ± 0.05	0.55

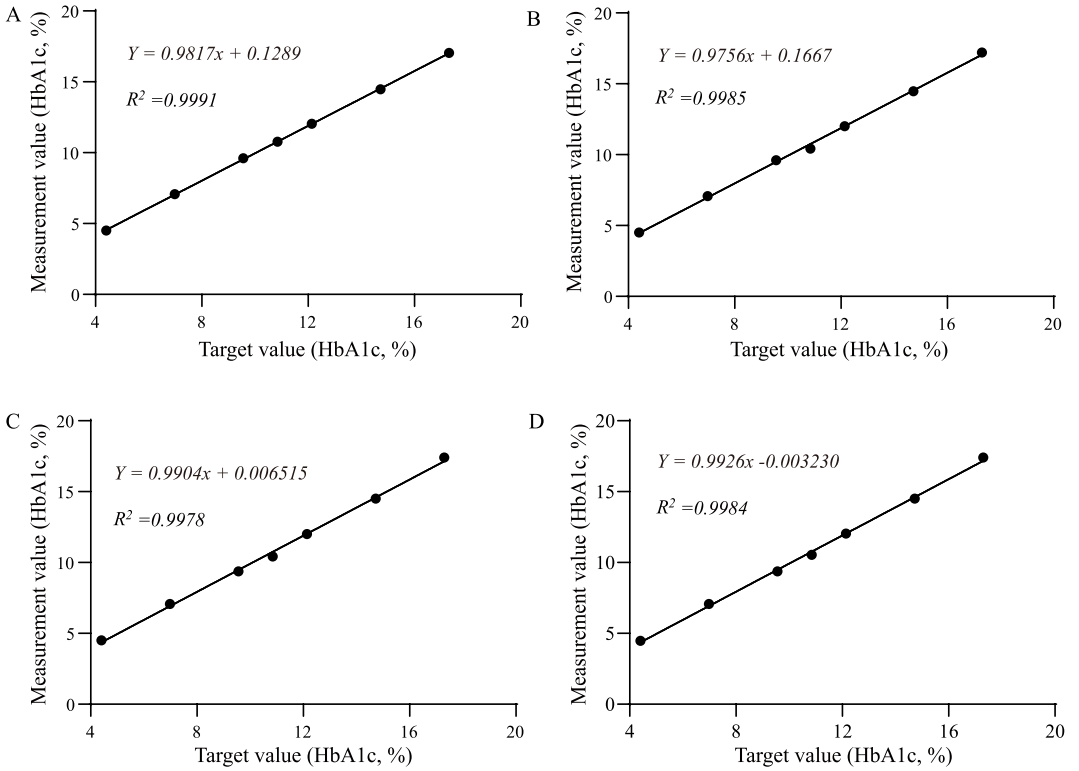

Fig. 4. The linearity of stability was evaluated by linear regression analysis to compare correlation between measured values and target values of HbA1c on the 3rd, 6th, 9th and 12th month, respectively. (A: 3rd month, B: 6th month, C: 9th month and D: 12th month).

Table 7
Characterization of potential interfering substances interference.

Interferent	Concentration	Sample	Testing result (HbA1c,%)		Bias (%)
			With interfering substance	Without interfering substance	
Triglycerides	20.0 mmol/L	L	5.11 ± 0.06	5.13 ± 0.05	0.39
		H	10.24 ± 0.05	10.26 ± 0.04	0.19
Hemoglobin	2.0 g/L	L	5.50 ± 0.04	5.48 ± 0.05	0.36
		H	13.52 ± 0.07	13.59 ± 0.07	0.51
Uric acid	20.0 mmol/L	L	5.20 ± 0.04	5.23 ± 0.05	0.57
		H	10.77 ± 0.04	10.72 ± 0.04	0.46
Ethanol	86.8 mmol/L	L	5.23 ± 0.05	5.21 ± 0.04	0.38
		H	8.66 ± 0.05	8.62 ± 0.05	0.46
Vitamin C	114.0 μmol/L	L	4.86 ± 0.05	4.88 ± 0.04	0.41
		H	10.86 ± 0.05	10.81 ± 0.05	0.46

hexahydrate are stable sodium salts that can be cleaned on chromatographic columns. Succinic acid was used to calibrate the pH. We believe that the formula of these mobile phase buffers was scientific and reasonable.

In this study, we evaluated the analytical performance of these mobile phase buffers. The results showed that the *CVs* of both the intra-assay and interassay quality control measurements, as well as those of the clinical blood samples, were less than 1.00 %. The guidelines for laboratory analysis of diabetes diagnosis recommend an intralaboratory CV $\leq$ 2.00 % and an interlaboratory CV $\leq$ 3.00 % [33,34]. The precision evaluations indicated that these mobile phase buffers possessed good reproducibility. However, precision is not sufficient to validate an excellent set of mobile phase buffers. Good accuracy is also needed to assess the difference between the measured and true values. All *RDs* were less than 1.00 %, lower than 6.00 %, which is a suggested criterion of the National Health Commission of China [33]. Adequate precision and accuracy are fundamentally important for HbA1c measurement. Linearity analysis revealed that this mobile phase buffer provided reliable results for a wide range of HbA1c levels (4.40%–17.30 % [25.00–166.00 mmol/mol]). Moreover, considering continuous measurement by automated machines, the true values of samples might be contaminated by adjacent samples. Therefore, we performed carryover experiments in our study. The carry-over percentage of the mobile phase buffers was 0.00 %. lower than the 1.00 % reported by Antonio Leon-Justel et al. using ARKRAY reagents [35]. Therefore, newly developed mobile phase buffers have wider linearity intervals, lower carry-over contamination rates, and better precision and accuracy than commercial buffers [36]. Moreover, these mobile phase buffer measurements are closely correlated with measurements of commercially available reagents in clinical blood samples.

Given the interference problems commonly encountered in clinical sample testing [37], we evaluated potential interfering substances such as triglycerides (up to 20.0 mmol/L), hemoglobin (up to 2.0 g/L), uric acid (up to 20.0 mmol/L), ethanol (up to 86.8 mmol/L) and vitamin C (up to 114.0 μmol/L). The results indicated that the mobile phase buffer had good anti-interference ability. However, we did not evaluate the interference of hemoglobin variants or other interfering substances. This is a limitation of our current study, and it will be considered in our future studies. The stability of the mobile phase buffers was also assessed, and the results indicated a stable quality for HbA1c over 12 months.

In summary, we systematically analyzed the performance of our mobile phase buffer compared with that of the commercially available buffer used for the ARKRAY system. The results demonstrated good performance for all evaluated parameters, including precision, linearity, accuracy, carryover, long-term stability, and anti-interference ability. The newly developed mobile phase buffer can be applied to the HA-8160 automatic glycohemoglobin analyzer for the HbA1c assay in clinical laboratories.

5. Research funding

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CRediT authorship contribution statement

Yuan Yu: Visualization, Validation, Methodology, Data curation. **Xiaoyun Zhang:** Validation, Project administration, Data curation. **Kai Lin:** Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Funding acquisition.

Declaration of competing interest

No conflicts of interest are declared.

Data availability

Data will be made available on request.

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Abbreviations

HbA1c	hemoglobin A1c
HPLC	High-Performance Liquid Chromatography
CV	Coefficient of variation
SD	Standard deviation
RD	relative deviation
CLSI	Clinical and Laboratory Standards Institute
NGSP	National Glycohemoglobin Standardization Program
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine.

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