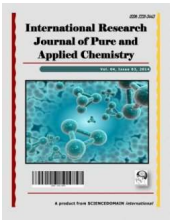

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Investigations on Synthesized *Azo* Compound, [4-((4-Hydroxynaphthalen-1-yl) Diazenyl) Benzoic Acid] (*p*-ABAαN), as an Acid-Base Indicator

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Authors' contributions

This work was carried in collaboration between all authors. Author CDA carried out the studies and wrote the protocol. Author EO preformed the statistical analysis and wrote draft of the manuscript with assistance from author CDA. Author WK supervised and directed the entire study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To evaluate the indicator properties of a synthesized *azo* compound to be used as a suitable substitute for standard indicators
Methodology: Compound, [4-((4-hydroxynaphthalen-1yl)diazenyl) benzoic acid](*p*-ABAαN) was synthesized using standard diazotization and coupling procedures. Initial evidence indicated that [4-((4-hydroxynaphthalen-1-yl) diazenyl benzoic acid] (*p*-ABAαN) exhibited sharp colour changes in acidic, neutral and alkaline pH media. Indicator properties in acid-base neutralization reactions were evaluated, and the results compared with that of phenolphthalein and methyl orange. The indicator, *p*-ABAαN was also used to assay Ibuprofen and validated upon comparison with official monograph (British Pharmacopoeia (2010) method). Results obtained were statistically analysed using *t*-test

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and One-Way Analysis Of Variance (One Way ANOVA) at 95% confidence level from Graph Pad Prism (version 6, 2012).
Results: The use of *p*-ABAαN in acid - base neutralization reactions involving strong acid / strong base and weak acid / strong base were experimentally verified and validated. Validation was also carried out for the use of *p*-ABAαN in titrimetric assay of Ibuprofen.
Conclusion: *p*-ABAαN [4-((4-hydroxynaphthalen-1-yl) diazenyl) benzoic acid] proved to be a suitable indicator for the titrimetric assay of Ibuprofen and in various acid-base titration experiments.

Keywords: Azo dye; indicator; methyl orange; phenolphthalein; titrimetry; validation.

1. INTRODUCTION

Titrimetry is widely used for quantitative analysis, and it is still the most frequently used method in pharmaceutical assays [1-2]. As an absolute method of analysis, it offers the advantage of determining the purity of a compound without a reference standard [1,3]. Titrimetric analysis requires the use of colour indicators for determination of end-points; examples of these indicators include methyl orange, methyl red, phenol red and phenolphthalein [1-4]. Indicators are needed in various institution laboratories for teaching and research purposes [5]. In the food and petroleum industries for instance, there is a need to constantly monitor acids and bases in both reaction mixtures and finished products, bringing into need titrimetric methods [6]. An example is the Kjeldahl method used for nitrogen determination in organic compounds [4,5]. In industries where routine and rapid analysis of raw materials intermediates and finished products are carried out for quality control purposes, indicators are very useful [7-9]. Previous studies in this area have shown that commercially used indicators originate either from natural sources [7,10-14] or from chemical synthesis [15-16]. Structurally, acid-base indicators are classified under three groups: phthaleins (e.g.phenolphthalein); sulphonaphthaleins (e.g. phenol red); and *azo* compounds (e.g. methyl orange) [4-5]. These compounds change from one colour to another as their chemical forms change as a result of changes in their chemical environment. The chemical changes may be due to excess of either H_3O^+ or OH^- ions, change in oxidation/reduction potential or a change in a physicochemical property of the indicator at the end point of the reaction. In an ideal titration, the visible end point coincides with the stoichiometric equivalence point [17]. However, a small difference occurs representing the titration or indicator error. Thus, indicator and experimental conditions selected for a titrimetric analysis should offer minimum difference between the visible end point and stoichiometric equivalence point [17]. The smaller the indicator error, the closer the outcome would be to the true value [4]. Hence accurate and reliable results are likely to be produced from such use. In this study, *p*-ABAαN [4-((4-hydroxynaphthalen-1-yl) diazenyl) benzoic acid] was synthesised and its indicator properties evaluated *p*-ABAαN would belong to *azo* group of indicators. We report herein our findings which revealed that *p*-ABAαN exhibits strong indicator properties.

2. MATERIALS AND METHODS

All chemicals used (that is, sodium nitrite, α-naphthol, *p*-aminobenzoic acid, conc. HCl, anhydrous NaOH pellets, methanol,) were of analytical grade and some obtained from BDH chemicals and/or Hopkins & Williams (H&W) laboratory chemicals. The *azo* dye *p*-ABAαN was synthesized and purified according to standard procedure [18]. Identification tests were carried out on the reagents before synthesis and analysis [19]. Thin Layer Chromatography (pre-coated silica plates of size (10 cm x 10 cm) was used to monitor the progress of the reactions, eluting with ethyl acetate:methanol (95:5). UV-Vis spectra of the synthesized compound were obtained on a Shimadzu t_{90+} UV-Vis Spectrophotometer (Japan) in 200 – 800 nm range in methanol, NaOH (0.1M) and acidified methanol. Infra-red spectrum was recorded using Shimadzu Fourier Transform Spectrometer (INTERSPECT) - (Japan) in the range of 400 - 4000cm^- using the KBr disc method. Synthesis of the indicator [4-((4-hydroxynaphthalen-1-yl) diazenyl) benzoic acid] (*p*-ABAαN) followed the general procedure employed which is diazotisation of primary aromatic amine followed by coupling with α-napthol, [19], (Scheme 1). This was then recrystallized to obtain a red brick solid product.

2.1 Screening of the Synthesised *Azo* Compound

p-ABAαN (0.05 g) was dissolved completely in ethanol (50 mL) and diluted to 100 mL with the

same solvent. 10 mL each of 0.1 M HCl, 0.1 M NaOH and deionized water were pipetted into separate 250 mL conical flasks. Five drops of the compound solution was added to the prepared solutions in the conical flasks at room temperature. Observation was made for colour changes in different pH conditions and *p*-ABAαN exhibited such varying changes in the acidic, basic and neutral media**.** Subsequently**,** the working or pH range was determined with a calibrated pH meter.

2.2 Potentiometric Titrimetric Analysis

A potentiometric set-up (a 50 mL burette, a 250 mL beaker with a magnetic stirrer) was employed. Titrations involving 0.1 M HCl and 0.1 M NaOH (strong acid-strong base) were carried out at room temperature using methyl orange and candidate indicator, *p*-ABAαN. Titrimetric end points as well as potentiometric equivalence points from the use of both indicators were determined [20]. Replicate titrations were carried out and indicator errors determined. The results were then analyzed statistically using One - Way ANOVA from Graph Pad Prism (version 6, 2012).

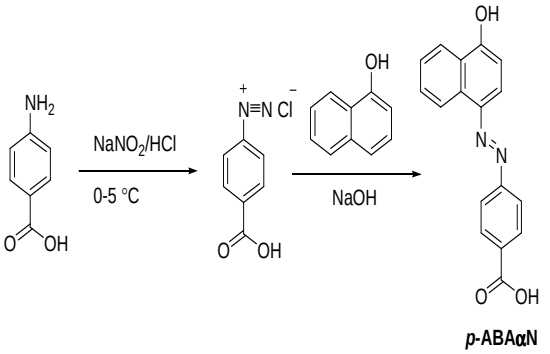
In a similar procedure, a solution of 0.1M CH_3COOH was titrated against 0.1M NaOH (weak acid-strong base) using phenolphthalein as the standard indicator and the candidate indicator, *p*-ABAαN. The equivalence points were again determined potentiometrically, as well as the end points. The results were then analyzed statistically using One–Way ANOVA from Graph Pad Prism (version 6, 2012).

2.3 Assay of Ibuprofen BP Powder

Titrimetric assay of Ibuprofen BP reference powder (0.450 g), was carried out by adopting the compendial method [8]. Five drops each of phenolphthalein and *p*-ABAαN were added to the respective prepared Ibuprofen solutions and titrated against with 0.1 M NaOH, as titrant. Replicate determinations were also carried out and results statistically analyzed using Graph Pad Prism (version 6, 2012).

2.4 Validation of the Indicator use

The use of *p*-ABAαN as an indicator in acid-base titrations involving HCl/NaOH and CH_3COOH/NaOH as well as in the assay of Ibuprofen was validated by evaluating parameters such as accuracy, repeatability, reproducibility and robustness [21]. Comparisons were made to standard or reference indicators as outlined in official monographs.



***p*-ABAαN**

Scheme 1. Synthesis of indicator *p*-ABAαN ([4-((4-hydroxynaphthalen-1-yl) diazenyl) benzoic acid])

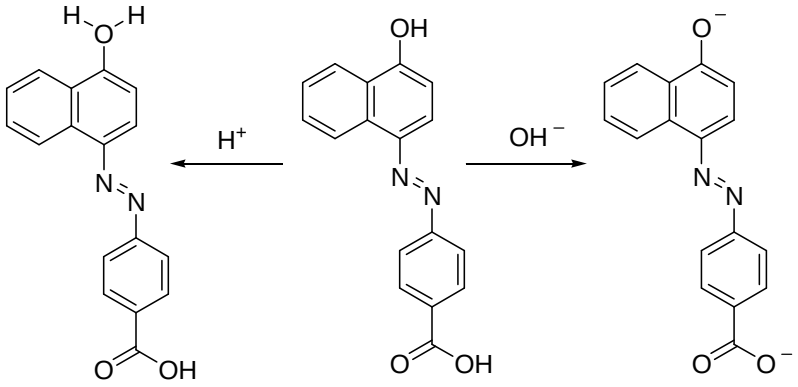
3. RESULTS AND DISCUSSION

3.1 Spectroscopic Analysis of Synthesized Dye

In this study, *p*-ABAαN, an *azo* compound was synthesized *via* the standard procedure [20] as illustrated in Scheme 1. The synthesized product was isolated in good yield of 72%. Spectroscopic analysis was carried out to ascertain the structure of the synthesized dye and the presence of functional groups in it.

3.2 Titrimetry

Observation of the synthesized dye to colour change in solutions led to the identification of *p*-ABAαN as an indicator. Drops of a 0.05% ethanolic solution of *p*-ABAαN gave a yellow colouration in acidic medium and turned pink in an alkaline medium. The phenolic OH group on the naphthol nucleus was protonated in an acidic medium, thereby reducing the electron cloud density and resulting in absorption at wavelength of 444 nm ((hypsochromic shift) (Fig. 1c) instead of 466 nm (Fig. 1a), indicated by observed yellow colouration. On the other hand, there was ionization of the phenolic OH and the carboxylic OH in alkaline medium (Scheme 2). This resulted in increased electron cloud density with resultant bathochromic shift (pink to red colouration, (λ 514 nm) (Fig. 1b). The pH range was determined to be 6.04 - 7.84.



Scheme 2. Effect of pH changes on the indicator *p*-ABAαN

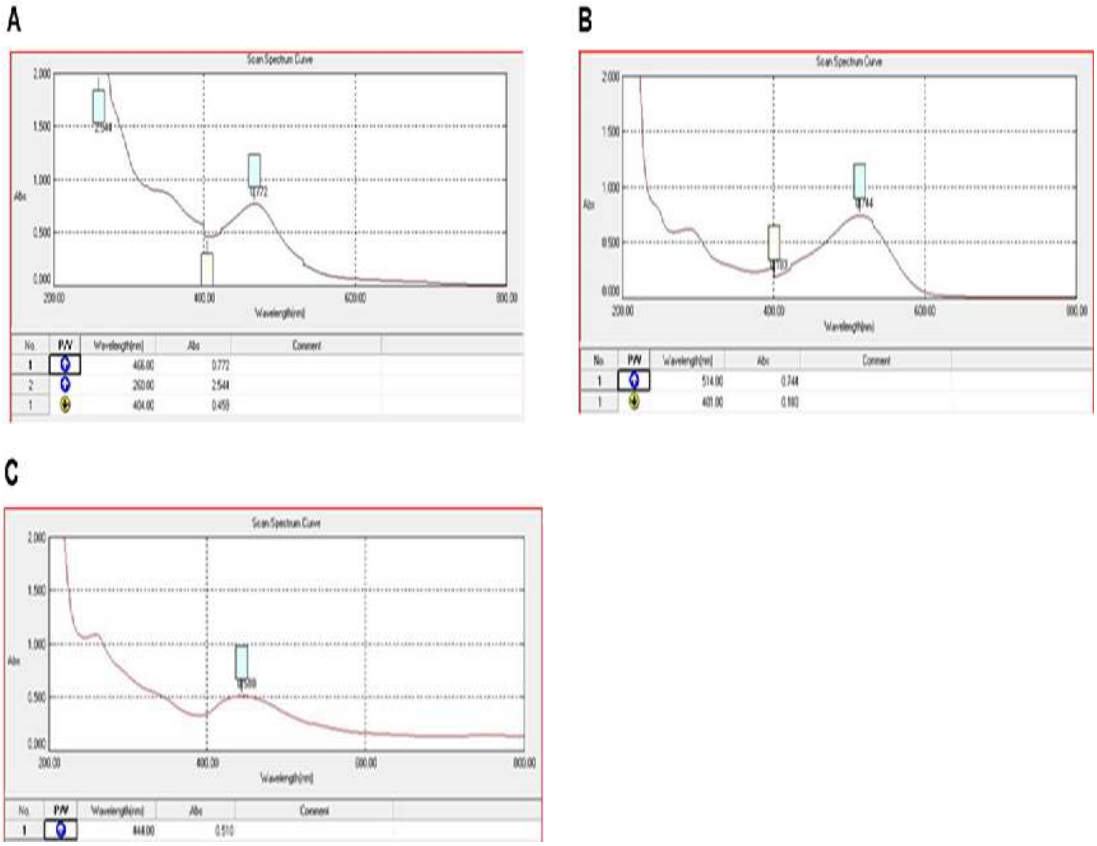


Fig. 1. UV spectral for p-ABAαN in different pH conditions

[A]- (Fig. 1a) Candidate dye in acid neutralized methanol. Absorption takes place at 466 nm. [B] (Fig. 1b) – Dye in basified methanol. Bathochromic shift leading to absorption at 514 nm. [C] (Fig. 1c)– Dye in acidified methanol. Hypsochromic shift resulting in absorption as a relatively lower wavelength, 444 nm

Titrimetric analysis involving 0.1MHCl/0.1M NaOH yielded a graph as shown below (Fig. 2). With NaOH solution serving as the analyte, the initial pH was determined to be alkaline (pH > 7). However, upon addition of acid (titrant), neutralization of the base with net excess H_3O^+ ions after equivalence point resulted in the rapid decline in pH [21]. The significant change in pH was thus, detected by the indicator, which due to the nature of the chemical environment, changed

colour (endpoint) .Similar results were obtained from titrations involving CH_3COOH/NaOH (weak acid/strong base). Furthermore, *p*-ABAαN was successfully used in place of phenolphthalein in the assay of Ibuprofen BP [8]. These results, obtained from *p*-ABAαN mediated analyses, were compared to that from standard indicators, methyl orange and phenolphthalein, as well as the potentiometric equivalence points respectively [21].

One-Way ANOVA analysis shown that there was no significant difference between the results obtained from methyl orange, *p*-ABAαN and the potentiometric equivalence points ($F_{2,27}$ = 3.124, p = 0.0602, N = 30;), (Fig. 2b). Similarly, results from phenolphthalein, *p*-ABAαN and potentiometric equivalence points were also shown to be significantly indifferent from each other ($F_{2,\ 27}$ = 1.500, p = 0.2411, N = 30;), (Fig. 2c).

Indicator errors were also determined for *p*-ABAαN and the two standard indicators under the conditions employed. It was observed from Student *t*-test analysis that, there was no significant difference between conventional indicator methyl orange and *p*-ABAαN. It was also shown that there was no significant difference between indicator errors of phenolphthalein and the dye, *p*-ABAαN.

3.3 Validation of Indicator Use

The accuracy of an analytical method is the closeness of test results obtained by such a method to the theoretical true value [18,22-24].

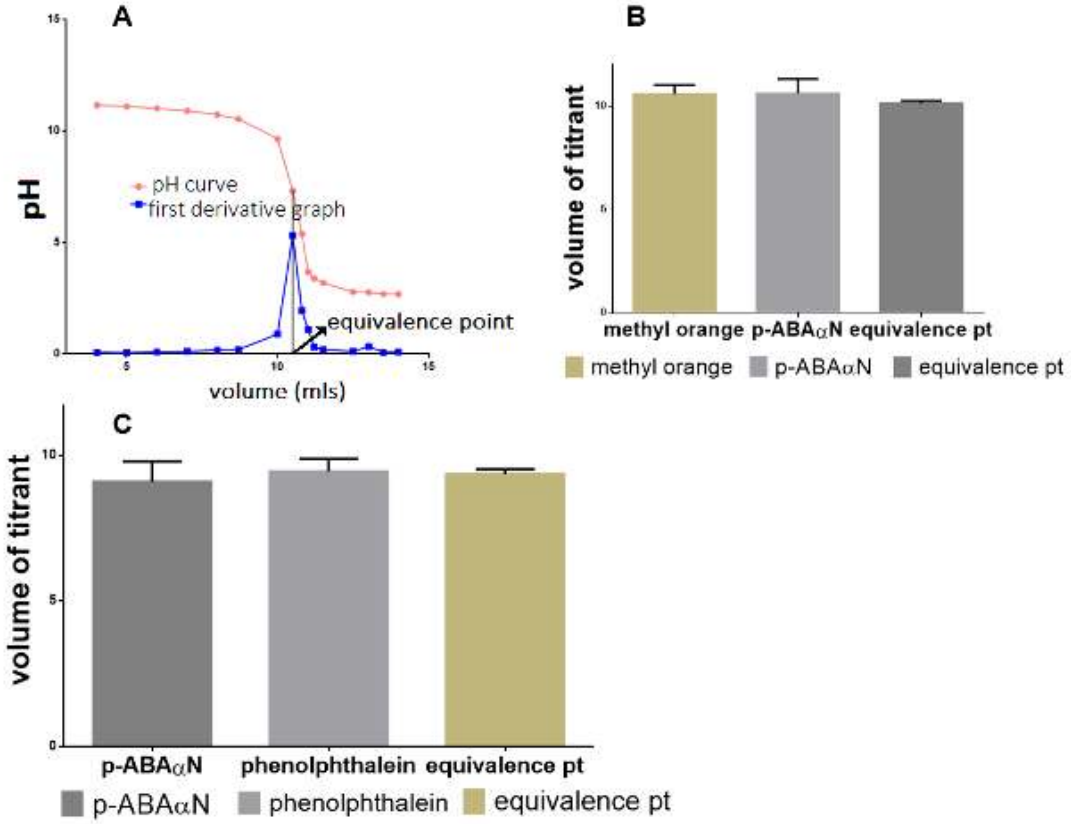


Fig. 2. Results from titrimetric analysis

*[A] – Determining the equivalence point from pH and first derivative curves for acid-base titration involving HCl and NaOH using p-ABAαN as indicator. [B] - End point volumes determined from HCl/NaOH titration using p-ABAαN and methyl orange as indicators. End points were then compared with potentiometric equivalence point from same titrations. Each bar represents Mean ± SEM of volume of titrant. [C] - End point volumes determined from CH_3COOH/NaOH titration using p-ABAαN and phenolphthalein as indicators. End points were then compared with potentiometric equivalence point from same titrations. Each bar represents Mean ± SEM of volume of titrant; *SEM (Standard error of mean)*

This was determined by comparing results obtained from the use of *p*-ABAαN with that of conventional indicators, that is, methyl orange for HCl/NaOH and phenolphthalein for CH_3COOH/NaOH. A paired two-tailed *t*-test analysis of stoichiometric endpoints from the use of *p*-ABAαN and methyl orange showed no significant difference (p = 0.1074, N = 30), (Fig. 3a). A similar analysis carried out on results from the use of *p*-ABAαN and phenolphthalein also failed to show significant difference (p = 0.0735, N = 30), (Fig. 3b). This shows that, results obtained from the use of *p*-ABAαN in both titrations were comparable to that produced with the widely accepted standard indicators. It also has an added merit of being able to replace both indicators. The accuracy of *p*-ABAαN function as indicator was further confirmed by calculating the indicator errors and comparing them, using paired *t*-test from Graph Pad Prism (version 6, 2012) at a confidence level of 95%.The results from analyses showed no significant difference between indicator errors of methyl orange and *p*-ABAαN (p = 0.0705, N = 30), as illustrated in (Fig. 3c) as well as phenolphthalein and *p*-ABAαN (p = 0.0659, N = 30), (Fig. 3d).

In the assay of Ibuprofen, percentage recovery calculated from the use of *p*-ABAαN ranged between 98.81% - 101.5% with a mean of 100.70% and a confidence interval of 100.1% - 101.4%. This further confirmed accuracy with the use of *p*-ABAαN in the assay of reference powder, as the confidence intervals complied with the acceptance criteria of 98.0 – 102.0% recovery as shown in Table 1.

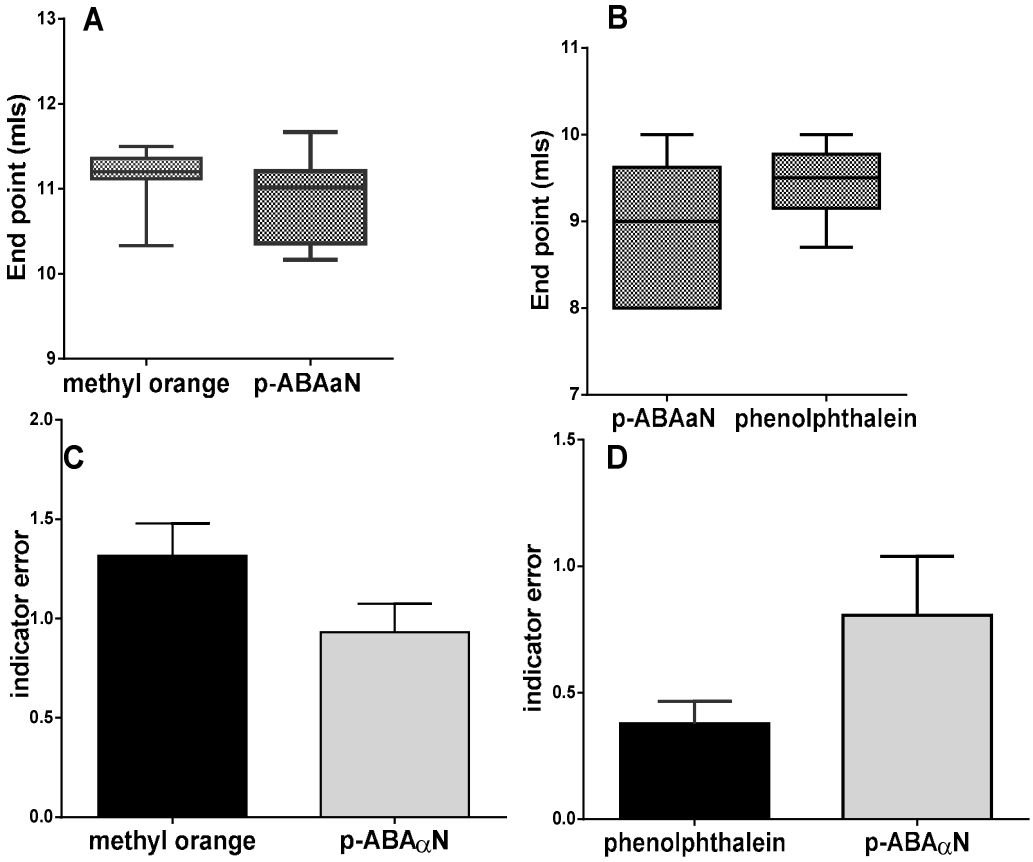


Fig. 3. Summary of stoichiometric end points and indicator errors from titrimetric analysis using p-ABAαN and standard indicators, methyl orange and phenolphthalein

*[A] (Fig. 3a) – Mean ± SEM of endpoint for titration between HCl and NaOH. [B] (Fig. 3b) – Mean ± SEM of endpoint for titration between CH_3COOH and NaOH. [C] (Fig. 3c) – Indicator errors for both p-ABAαN and methyl orange in the titration between HCl and NaOH. [D] (Fig. 3d) - Indicator errors for both p-ABAαN and phenolphthalein in the titration between CH_3COOH and NaOH; * SEM - Standard error of mean*

Table 1. Percentage recovery of Ibuprofen BP using p-ABAαN as indicator

Percentage purity		
Phenolphthalein	***p*-ABAαN**	**Percentage recovery**
97.74	99.10	101.39
98.70	100.20	101.52
97.75	98.60	100.87
99.81	100.10	100.29
98.30	99.50	101.22
95.50	96.79	101.35
99.08	97.90	98.81
100.04	100.99	100.95
100.25	100.10	99.85
98.60	99.82	101.24
Mean ± SEM = 100.70%±0.2722		

Acceptance Criteria = 98.0% - 102.0%

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. It is expressed either as standard deviation (SD) or relative standard deviation (RSD) of a series of determinations [25]. The precision of the analytical procedure with the use of *p*-ABAαN was established by testing for repeatability *via* statistically analysing results obtained from titrations involving HCl/NaOH and CH_3COOH/NaOH. The RSD at the working concentration of the indicator (0.05%) for ten replicate titrations were found to be 0.49% and 1.09% respectively, which complied with the acceptance criteria of ≤ 2.0% as shown in Table 2. Similar determinations were carried out for assay of Ibuprofen and RSD was observed to be 0.81%, which also complied with the acceptance criteria.

Intermediate precision was also tested statistically by analyzing test results obtained firstly, on different days by same analyst, and then secondly by two independent analysts on the same day. The RSD for inter-day precision for the three different analysis were all observed to be less than 2.0%, complying with the acceptance criteria of ≤ 2.0% (Table 3). This signified a high level of closeness and consistency of test results over different days. In further confirmation of precision, these test results were subjected to One - Way ANOVA analysis and it was observed that there were no significant differences; ($F_{2,27}$ = 2.661, p = 0.0882, N = 30) for HCl/NaOH, (strong acid/strong base) titrations ($F_{2,27}$ = 0.4013, p = 0.6734, N = 30) for CH_3COOH/NaOH (weak acid/strong base) titrations) and ($F_{2,27}$ = 0.05377, p = 0.9478, N = 30) for assay of Ibuprofen BP. In the analysis of test results from the two independent analysts, it was also observed that the RSD for the three analysis were less than 2.0% (Table 4). A paired two-tailed Student *t*-test analysis of the results also showed no significant differences in results from the two analysts; (t = 0.9221, df = 10, p = 0.3782, N = 11) for HCl/NaOH, (t = 0.8964, df = 10, p = 0.3911, N = 11) for CH_3COOH/NaOH and (t = 0.2345, df = 10, p = 0.8193, N = 11) for assay of Ibuprofen BP.

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present [18]. Proving specificity involves the establishment of the presence of *p*-ABAαN in the indicator solution and attributing the outcome of analysis (end points) to it and not the solvent (that is, ethanol). A 0.05% solution of *p*-ABAαN was prepared in ethanol. In the quest to prove the lack of influence of the ethanol (as the solvent) on the results produced by the indicator, similar volumes (10 mL ±0.1) of methanol and indicator were employed. 5 drops of each ; ethanol and *p*-ABAαN solution were independently added to separate conical flasks containing 10.00 ml of 0.1 M NaOH, 0.1 M HCl and 0.1 M CH_3COOH and their colour changes were noted. Titrations were carried out using 0.1 M HCl, 0.1 M NaOH respectively and colour changes again noted and recorded. It was observed from Table 5 below that, drops of ethanol added to the analyte solution failed to effect change in colour of the analyte solution. It was also not able to visually predict the end of the stoichiometric reaction between the analyte and the titrant. The observation of colour changes (end point) during the titrations indicates that the results were only

attainable by virtue of the presence of the indicator *p*-ABAαN. Thus the activity of *p*-ABAαN is specific for the three stoichiometric reactions considered in this study.

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters [18,23].

Table 2. Equivalence points and purity determinations in the evaluation of repeatability

HCl /NaOH (mL)	CH_3COOH/NaOH (mL)	Assay of pure Ibuprofen powder (Purity) (%)
11.5	9.0	99.100
11.5	8.8	100.20
11.5	8.8	99.60
11.4	9.0	100.10
11.5	8.9	99.50
11.5	9.0	98.79
11.6	9.0	100.90
11.5	9.1	100.99
11.5	9.0	99.10
11.6	8.9	100.82
Mean = 11.51 ± 0.018	Mean = 8.95 ± 0.031	Mean = 99.91 ± 0.257
RSD = 0.49%	RSD = 1.09%	RSD = 0.81%

Table 3. Results from same analyst on different experimental days

HCl/NaOH (mL)			CH_3COOH/NaOH (mL)			Assay of pure Ibuprofen powder (Purity) (%)		
Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
11.20	11.00	10.90	9.00	9.10	9.10	99.10	101.74	99.87
11.00	11.10	10.80	9.10	9.00	9.10	100.20	99.70	100.21
11.00	11.30	11.30	9.20	9.00	9.00	99.60	99.75	99.95
11.10	11.20	11.10	9.00	9.10	9.00	100.10	99.81	99.83
11.00	11.30	10.90	9.10	9.10	9.20	99.50	98.30	100.59
11.00	11.50	11.20	9.00	9.20	9.10	98.79	98.94	98.99
11.10	11.40	11.00	9.10	9.10	9.00	100.90	100.08	99.75
11.10	11.20	11.20	9.10	9.00	9.00	100.99	99.84	100.03
11.00	11.10	11.30	9.20	9.10	9.10	99.10	99.25	99.41
11.00	11.00	11.40	9.20	9.00	9.20	100.82	100.60	99.77
Mean = 11.12±0.0302			Mean = 9.08±0.0136			Mean = 99.85±0.1333		
RSD = 1.49%			RSD = 0.82%			RSD = 0.73%		

Table 4. Results from different analysts on the same day

HCl/NaOH (mL)		CH_3COOH/NaOH (mL)		Assay of pure Ibuprofen powder (Purity) (%)	
Analyst 1	Analyst 2	Analyst 1	Analyst 2	Analyst 1	Analyst 2
11.00	11.10	9.10	9.00	98.79	99.60
11.00	11.00	9.20	9.10	100.90	100.10
11.10	10.90	9.20	9.00	100.99	99.50
11.10	10.80	9.10	9.10	99.10	99.70
11.00	11.30	9.00	9.00	99.83	99.75
11.00	11.00	9.10	9.20	100.59	99.81
11.00	11.00	9.20	9.10	98.99	99.84
11.10	11.10	9.10	9.00	99.75	99.25
11.30	11.20	9.00	9.10	100.03	100.60
11.20	11.00	9.10	9.10	99.41	99.87
11.30	11.20	9.00	9.10	98.94	99.95
Mean = 11.08±0.0279		Mean = 9.086±0.0152		Mean = 99.79±0.1280	
RSD = 1.18%		RSD = 0.78%		RSD = 0.60%	

It provides an indication of its reliability during normal usage. In testing for robustness, the first adopted approach involved preparing 50%, 100% and 150% concentrations corresponding to 0.025%, 0.05% and 0.1% of the indicator respectively and adding 5 drops of each separately to analyte solutions for analyses. The second approach was by using 3 drops, 5 drops and 7 drops of the indicator solution for the analyses. Relative Standard deviations (RSD) worked out on the results obtained from the titrations showed that, they were all less than 2.0% (Tables 6 & 7) and thus, complied with the acceptance criteria. It was also observed that altering a condition in the original method did not produce significantly different results. From One – Way ANOVA analysis, the following results were produced when the concentration of the indicator solution was altered; ($F_{2,15}$ = 0.7609, p = 0.4845, N = 18) for HCl/NaOH, ($F_{2,15}$ = 0.6481, p = 0.5371, N = 18) for CH_3COOH/NaOH and for Ibuprofen assay ($F_{2,15}$ = 0.1924, p = 0.8270, N = 18). In varying the number of indicator drops, the following statistical results were also obtained; ($F_{2,15}$ = 0.5072, p = 0.6121, N = 18) for HCl/NaOH, ($F_{2,15}$ = 0.3571, p = 0.7055, N = 18) for CH_3COOH/NaOH and Ibuprofen assay ($F_{2,15}$ = 0.1407, p = 0.7055, N = 18). It could therefore be established that the use of the indicator was robust as changes in some developed method conditions did not significantly alter the test results, which also were found to be very precise.

The stability of the indicator solution is very important since it gives an idea of the time frame within which a prepared solution could be safely and confidently used for the intended purpose. The stability of *p*-ABAαN was determined by employing freshly prepared 0.05% indicator solution in HCl/NaOH titrations over a twelve-week duration of acid-base analysis. Replicate titrations were carried out on a weekly basis and the calculated RSDs were shown to be less than 2.0%, complying with the acceptance criteria (Table 8). One – Way ANOVA analysis also demonstrated an absence of significant difference ($F_{11,24}$ = 0.8138, p = 0.6273, N = 36) (Fig. 4).

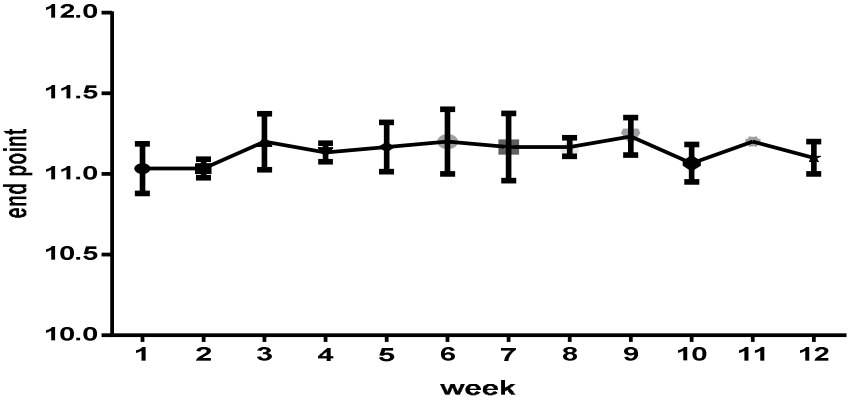


Fig. 4. End points obtained from a three month study of the use a freshly prepared 0.05% p-ABAαN solution

Table 5. Colour of indicator and solvent in different solutions

	***p*-ABAαN**			**Ethanol**		
	NaOH (analyte)/ HCl (titrant)	HCl (analyte)/ NaOH (titrant)	CH_3COOH (analyte)/ NaOH (titrant)	NaOH (analyte)/ HCl (titrant)	HCl (analyte)/ NaOH (titrant)	CH_3COOH (analyte)/ NaOH (titrant)
Colour of indicator before titration	Pink	Yellow	Yellow	Colourless	Colourless	Colourless
Colour of indicator after titration	Yellow	Pink	Pink	Colourless	Colourless	Colourless

Table 6. Results produced from altering concentration of indicator

HCl/NaOH (mL)			CH_3COOH/NaOH (mL)			Assay of Ibuprofen (%)		
0.025%	**0.05%**	**0.1%**	**0.025%**	**0.05%**	**0.1%**	**0.025%**	**0.05%**	**0.1%**
11.10	11.20	11.20	9.00	9.20	9.10	99.75	99.75	99.10
11.00	11.10	11.00	9.10	9.00	9.00	100.03	99.81	100.20
11.00	11.00	11.00	9.10	9.10	9.20	99.41	98.30	99.60
11.00	11.10	11.00	9.20	9.10	9.10	100.08	99.83	100.10
11.10	11.00	11.10	9.00	9.00	9.20	99.84	100.59	99.50
11.00	11.10	11.00	9.10	9.00	9.10	99.25	98.99	99.70
Mean = 11.03±0.0211	Mean = 11.08±0.0307	Mean = 11.05±0.0342	Mean = 9.083±0.0307	Mean = 9.067±0.0333	Mean = 9.117±0.0307	Mean = 99.73±0.1363	Mean = 99.54±0.3241	Mean = 99.70±0.1653
RSD = 0.47%	RSD = 0.68%	RSD = 0.47%	RSD = 0.83%	RSD = 0.90%	RSD = 0.83%	RSD = 0.33%	RSD = 0.80%	RSD = 0.41%

Table 7. Results from altering number of drops of indicator

HCl/NaOH (mL)			CH_3COOH/NaOH (mL)			Assay of Ibuprofen (%)		
3 drops	**5 drops**	**7 drops**	**3 drops**	**5 drops**	**7 drops**	**3 drops**	**5 drops**	**7 drops**
11.20	11.10	11.00	9.00	9.10	9.10	98.79	99.84	100.99
11.10	11.00	11.00	9.10	9.20	9.10	100.90	99.25	99.10
11.00	11.00	11.00	9.10	9.00	9.20	99.50	100.03	99.75
11.00	11.10	11.10	9.10	9.00	9.00	99.70	99.41	99.81
11.10	11.30	11.00	9.20	9.20	9.10	99.83	99.60	98.99
11.00	11.00	11.10	9.10	9.10	9.00	100.59	100.10	99.75
Mean = 11.07±0.0333	Mean = 11.08±0.0477	Mean = 11.03±0.0211	Mean = 9.10±0.0258	Mean = 9.10±0.0365	Mean = 9.067±0.0333	Mean = 99.89±0.3115	Mean = 99.71±0.1396	Mean = 99.73±0.2909
RSD = 0.74%	RSD = 1.05%	RSD = 0.47%	RSD = 0.83%	RSD = 0.90%	RSD = 0.83%	RSD = 0.76%	RSD = 0.34%	RSD = 0.71%

Table 8. Results on stability of p-ABAαN

Week		Determinations			Mean ± SEM	RSD (%)
		First (mL)	Second (mL)	Third (mL)		
1	1st month	11.20	11.00	10.90	11.03±0.088	1.38
2		11.00	11.10	11.00	11.03±0.033	0.52
3		11.00	11.30	11.30	11.20±0.100	1.55
4		11.10	11.20	11.10	11.13±0.033	0.52
5	2nd month	11.00	11.30	11.20	11.17±0.088	1.37
6		11.00	11.40	11.20	11.20±0.116	1.79
7		11.10	11.40	11.00	11.17±0.120	1.86
8		11.10	11.20	11.20	11.17±0.033	0.52
9	3rd month	11.30	11.10	11.30	11.23±0.067	1.03
10		11.00	11.00	11.20	11.07±0.067	1.04
11		11.20	11.20	11.20	11.20±0.000	0.00
12		11.20	11.00	11.10	11.10±0.058	0.90

4. CONCLUSION

It has been established that, [4-((4-hydroxynaphthalen-1-yl) diazenyl) benzoic acid] (*p*-ABAαN) can be used as a suitable indicator for the titrimetric assay of Ibuprofen. It can also play a dual role as an alternative indicator to both methyl orange and phenolphthalein as evidenced in strong acid/strong base and weak acid/strong base titrimetric analysis. The presence of *p*-ABAαN in laboratories could be a suitable substitute for both methyl orange and phenolphthalein, reducing cost and laboratory storage space. Multi-kilogram commercial production to ensure availability of *p*-ABAαN to support various analytical works in teaching and research laboratories remains our ultimate goal.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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