Research Article

Acid-Base Indicator Properties of Synthesized Phenylhydrazones: 4-(2-(2,4-Dinitrophenyl) Hydrazono) Methyl)-2 Methoxy Phenol and 4-(2-(2,4-Dinitrophenylhydrazono) Methyl) Benzene-1,3-diol

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4-(2-(2,4-Dinitrophenyl) hydrazone) methyl)-2 methoxy phenol (IND-X) and 4-(2-(2, 4-dinitrophenylhydrazono) methyl) benzene-1,3-diol (IND-Y) were synthesised by condensation reactions with 2,4-dinitrinophenylhydrazine and were observed to possess acid-base indicator properties. The study was conducted to explore and verify their use in different analytical situations and, consequently, validate them. Analysis of their use in acid-base titrations between strong acids/strong bases and weak acids/strong bases as well as pharmaceutical applications in the assay of ibuprofen pure powder and batch of ibuprofen tablets (400 mg) using standard methods in the British Pharmacopoeia (2020) was all considered. The validations were performed by the International Conference on Harmonisation of Technical Requirements for the registration of Pharmaceuticals for Humans Q2 (R1) guidelines. The outcomes were statistically compared with existing conventional analytical approaches. Titrimetric data obtained were analyzed statistically by Student’s t-test and one-way ANOVA at a 95% confidence level. IND-X and IND-Y were shown to be similar in behaviour to phenolphthalein, methyl orange, and methyl red as visual indicators after preliminary evidence of sharp colour changes in acid, alkaline, and neutral pH solutions. The use of the two indicators in the assay of ibuprofen powder and tablets has also been established. The validation parameters considered were found to be consistent with respective acceptance criteria for analytical purposes. IND-X and IND-Y are therefore proposed as alternative acid-base indicators to methyl red, methyl orange, and phenolphthalein for routine volumetric analysis in students’ laboratory experiments. They also serve as alternative indicators to phenolphthalein for the titrimetric analysis of ibuprofen-based samples and products.

1. Introduction

The technique of titrimetric quantitative analysis is broadly based on volumetric, gravimetric analysis, and biomedical 2 analytical chemistry [1]. The titrimetric analysis is one of the most used and relevant analytical techniques in pharmaceutical analysis. The speed of analysis, the high precision determination of the purity of compounds in the absence of reference standards, instantaneous completion of reactions, and monitoring of endpoints through indicators and electrometric methods have made titrimentry still useful in several applications, including pharmaceutical analysis [1].

An indicator as used in titrimetry is a chemical substance sensitive enough to display a clear change incolour very close
to the point in the ongoing titration process at which equimolar quantities of the analyte and titrant have almost virtually reacted with each other [2, 3]. Generally, chemical indicators may be classified into acid-base, oxidation-reduction, complexometric, adsorption, and chemiluminescent indicators [4]. The pH indicators are acid-base indicators, mostly weak acids such as methyl orange, phenolphthalein, and phenol red, and are coloured differently in their dissociated and associated forms. Acid-base titrations depend on the neutralization between an acid and a base when mixed in solution. The acid-base indicator indicates the endpoint of the titration by changing colour. The colour change of the indicator is pH-dependent, as it affects the dissociation of the indicator compound. Consider an indicator which is a weak acid, with the formula HIn. At equilibrium, the following equilibrium equation is established with its conjugate base: HIn + H2O = H2O+ + In, where HIn refers to the indicator colour in an undissociated state and In refers to the indicator colour in dissociated condition. The indicator exhibits different halochromic properties under different dissociation conditions, and this tends to affect its colouration in the presence of analytes being investigated. The indicator endpoint and the equivalence point are not the same because the equivalence point is determined by the stoichiometry of the reaction, while the endpoint is just the colour change from the indicator. However, the choice of a suitable indicator for titration depends on its ability to minimise the difference (or indicator error) between the endpoint and the nominal equivalence point [5, 6]. Earlier works show that p-nitrophenyldiazones do possess halochromic properties, and this makes them good candidates as indicators. For example, the potassium salt of benzaldehyde p-nitrophenyldiazone was observed to change colour from yellow to red as pH increased from below 11.3 to 11.7 and above [7]. Also, salicylaldehyde phenyldiazones have been used as colour indicators in the titration of organometallic agents including Grignard reagents [8]. Similarly, p-nitrophenyldiazone of pyridine-aldehyde has also been proposed as an alkali indicator [9]. The halochromic effect of nitrophenyldiazones is partly attributed to the presence of the nitro group and partly to the nature of the electrophilic substituents attached, which tend to reduce the electron density of the molecule, making it possible for the release of proton [9]. As a result, several derivatives of nitrophenyldiazone have been explored for their acid-base indicator properties, and these include but are not limited to crotonaldehyde- and acrylaldehyde p-nitrophenyldiazones, furfurylidene- and 5-nitro-2-furfurylidene, p-nitroalkyldiazones, and picolinealdehyde [9].

Following up on the previous synthesis and antimicrobial studies on phenyldiazones [10], we hereby report the validation of the strong colour indicator property of two of those compounds in acid-base titrations.

2. Experimental

2.1. Materials and Chemicals. The materials used in the study included silica gel (70 : 230 mesh size) (Merck, US), silica gel precoated TLC plates (Merck, Germany), ibuprofen BP powder (98% w/w), and ibuprofen tablets (400 mg) (Ernest Chemists, Ghana). The chemicals employed were ethyl acetate, petroleum ether, concentrated hydrochloric acid (36% w/v), sodium hydroxide pellets, 2,4-dinitrophenyldiazine, vanillin (4-hydroxy-3-methoxy benzaldehyde), 2,4-dihydroxy benzaldehyde, Analar anhydrous sodium carbonate (99.5% w/w), methanol, Analar sulphamic acid (99% w/w), and commercial acetic acid (36% w/v) which were all sourced from Fisher Scientific, United Kingdom.

2.2. Synthesis of the Phenylhydrazones. The two compounds, 4-(2-(4-dinitrophenyl) hydrazone) methyl-2 methoxy phenol (IND-X) and 4-(2-(4-dinitrophenyl) hydrazone) methyl benzene-1,3-diol (IND-Y), were synthesised following the synthetic schemes as reported [10] and as illustrated in Figure 1.

2.2.1. Synthesis of 4-(2-(4-Dinitrophenyl) Hydrazono) Methyl)-2 Methoxy Phenol. IND-X was prepared from the reaction between 2,4-dinitrophenyldiazine (0.78 g, 3.93 mmol, 1 eq.) dissolved in methanol (10 mL) with 4-hydroxy-3-methoxy benzaldehyde (vanillin) (0.50 g, 1.04 eq., 4.09 mmol) in an acidified medium (concentrated H2SO4, 98% v/v, 2 mL) and maintained in an iced bath (0°C) with stirring for at least 24 hours. The reaction was monitored with TLC plates, and upon completion, the product was purified by recrystallization, and the identity of the compound was confirmed by comparing its melting point and spectroscopic data with the literature [10].

2.2.2. Synthesis of 4-(2-(4-Dinitrophenyl) Hydrazono) Methyl Benzene-1,3-diol. IND-Y on the other hand was produced from the reaction between 2,4-dinitrophenyldiazine (0.69 g, 3.48 mmol, 1 eq.) dissolved in methanol (10 mL) with 2,4-dihydroxy benzaldehyde (0.50 g, 1.04 eq., 3.62 mmol) in an acidified medium (concentrated H2SO4, 98% v/v, 2 mL) and maintained in an iced bath (0°C) with stirring for at least 24 hours. The reaction was also monitored with TLC plates, and upon completion, the product was purified by recrystallization, and the identity of the compound was confirmed by comparing its melting point and spectroscopic data with the literature [10].

2.3. Characterisation of Synthesised Phenylhydrazones

2.3.1. 4-(2-(4-Dinitrophenyl) Hydrazone) Methyl)-2 Methoxy Phenol. IND-X (0.89 g, 75%) presented as an orange solid. RF (Pet. ether 70%: EtOAc 30%): 0.63. Mpt: 267–270°C. UV-Vis (MeOH) λmax: 218 nm and 394 nm. Infrared (neat) ucm⁻¹: 3360 (OH), 3253 (NH), 3080 (C=CH); 1H NMR (400 MHz, CDC13) δH 11.50 (1H, s, NH), 10.02 (1H, H-C4', s, ArOH), 9.62 (1H, H-C3, s, ArH), 8.75 (1H, H-C5, s, ArH), 8.68 (1H, H-C7, s, N=CH), 8.30 (1H, H-C6, d, J = 4.0, ArH), 7.90 (1H, H-C2', d, J = 4.0, ArH), 7.60–7.80 (1H, H-C6', d, J = 8.0, ArH), 6.35–6.38 (1H, H-C5', d, J = 12.0, ArH), (3H, H-C4', Ar-OCH3). 13C NMR (400 MHz, CDC13) δc 150.0, 150.2, 148.5, 144.6, 136.9, 130.2,
2.3.2. 4-(2-(2,4-Dinitrophenylhydrazono) Methyl) Benzene-1,3-diol. IND-Y (0.83g, 75%) was produced as a dark red solid. Mpt: 270–272°C; UV-Vis (MeOH) \(\lambda_{\text{max}}: 218\text{nm and } 403\text{nm}\); Infrared (neat) \(\nu_{\text{max}}: 3350, 3090, 1620, 1570\text{cm}^{-1}\).

dH(400MHz;CDCl3):11.52(1H(C1′), s, ArOH), 10.02(1H (C1), s, NH), 9.96 (1H (C3′), s, ArOH), 8.80 (1H (C3), s, ArH), 8.78 (1H, s, N\(\text{CH}\)), 8.30–8.36 (1H (C5), d, \(J=12.0,\) ArH), 7.92–7.96 (1H (C6), d, \(J=8.0,\) ArH), 7.50–7.56 (2H (C2′, C5′), m, ArH), 6.35 (1H (C4′), d, \(J=8.0,\) ArH). δc 160.2, 157.2, 148.0, 145.7, 136.2, 130.2, 129.2, 127.8, 122.4, 116.0, 112.2, 106.0, 102.2 (Ar). These parameters were also consistent with the literature [10].

2.4. Screening of the Synthesised Compounds for Indicator Property. The two indicators, IND-X and IND-Y (0.05g), were each dissolved completely in ethanol (50mL) and diluted to the 100 mL mark with the same solvent in a volumetric flask to obtain a final concentration of 0.05% w/v. Indicator solutions of different pHs were prepared using acetate (pH = 3.0), phthalate (pH = 7.00), and phosphate buffers (pH = 10.0). Five drops of each indicator were added to the prepared solutions at room temperature, and observations were made for colour changes.

2.5. IND-X and IND-Y as Alternative Visual Indicators for Conventional Titrations. The titrimetric analyses considered included strong base vs. strong acid and strong base vs. weak acid titrations. In the strong base vs. strong acid titrimetric investigations, standardised 0.5 M NaOH (10mL) was titrated with standardised 0.5 M HCl using IND-X and IND-Y as test visual indicators. Methyl orange, methyl red, and phenol red as standard indicators were also used, and their results were compared with that of the test indicators. In the strong base vs. weak acid titrations, standardised 0.5 M NaOH (10mL) was titrated with standardised 0.5 M CH₃COOH using IND-X and IND-Y as test visual indicators. Phenolphthalein as a standard indicator was used, and its results were compared with that of the test indicators.

The titrimetric endpoints and potentiometric equivalence points from the indicators were determined, and their respective indicator errors were calculated. Replicate determinations were made, and the data obtained were statistically analyzed by one-way ANOVA. The same analyses were repeated with titrations involving 0.5 M NaOH (solution (10mL) and 0.5 M CH₃COOH (strong base/weak acid) using phenolphthalein as the standard indicator and the two test indicator solutions in triplicate.

2.6. IND-X and IND-Y as Alternative Visual Indicators for Titrimetric Assay of Ibuprofen BP Powder and Tablets. The British Pharmacopoeia method was used to assay ibuprofen BP starting material (0.4000g) and formulated tablets (equivalent weight containing 400mg ibuprofen) [11]. The prepared ibuprofen solutions were titrated with a standardised solution of 0.1 M NaOH (titrant) (\(f = 0.9980\)), using IND-X, IND-Y, and phenolphthalein as indicators. Replicate analyses were carried out, and the results were analyzed statistically.

2.7. Validation of Indicator Property. The use of IND-X and IND-Y in titrations involving NaOH/HCl (strong base/strong acid) and NaOH/CH₃COOH (strong base/weak acid), and in the assay of ibuprofen, BP powder and tablets...
(400 mg) were validated according to the International Conference on Harmonisation (ICH) Q2 (R1) guidelines [12].

3. Results and Discussion

3.1. Spectroscopic Characterisation of the Compounds.

There were two absorption peaks in the UV-visible spectrum at a wavelength maximum of 218 nm and 394 nm as also reported in the literature [10]. The infrared spectrum revealed the presence of a broad band at 3360 cm\(^{-1}\) reported in the literature [10]. Bands at 3253 cm\(^{-1}\) and 3080 cm\(^{-1}\) indicated the presence of a secondary amine group and an sp\(^2\)-hybridised carbon, respectively. In the \(^1\)H NMR spectrum, the formation of the imine bond was confirmed by the presence of a singlet resonating at 8.68 ppm, whilst the other peaks also corroborated with that reported by [10].

3.2. Acid-Base Indicator Properties of the Compounds. The condensation reactions between 4-hydroxy-3-methoxy benzaldehyde and 2,4-dihydroxy benzaldehyde, with 2,4-dinitrophenylhydrazine, produced orange and dark red coloured products which absorbed light in the UV-Vis region. This phenomenon is due to their extended conjugations as a result of the introduction of extra chromophores after the reactions, which result in their wavelengths of absorption being in the visible region of the electromagnetic spectrum.

In an alkaline medium, both IND-X and IND-Y are ionised or deprotonated, and this is illustrated in Figure 2. The presence of the hydroxy groups in both compounds provides the chemistry behind their ionisation. The extra electron gained as a result of the ionisation increases the electron density of the benzene-bearing hydroxy group, and this results in a bathochromic shift to 394 and 403 nm, respectively (Figure 3). Consequently, this translates into changes in colour for the ethanolic solutions of the compounds in acid, neutral, and alkaline media (Table 1). In an acidic medium, IND-X and IND-Y become the phenolic hydroxyl group tethered to the benzene ring and are protonated, resulting in a decreased electron cloud density and exhibiting a yellow colour associated with a hypsochromic shift at 218 and 210 nm, respectively.

3.3. Titrimetric Analysis with IND-X and IND-Y.

Titrimetric analyses were carried out with the test indicators, IND-X and IND-Y, at 0.05% (w/v) concentration of each in ethanol. In the titration involving 0.5 M NaOH and 10.00 mL of 0.5 M HCl, IND-X was observed to transition from a yellowish colour associated with the use of the indicators was defined as the closeness of their titrimetric endpoints with that of conventional indicators; and this was determined by comparing results obtained from the use of IND-X and IND-Y with that of methyl orange and methyl red for titrations involving 0.1 M HCl/0.1 M NaOH, phenol red, and phenolphthalein for titrations involving 0.1 M CH\(_3\)COOH/0.1 M NaOH and phenolphthalein for the titrimetric assay of ibuprofen.

3.4. Validation of Use of Indicators. In validating the use of the indicators in the above-described titrimetric applications, the parameters, specificity, accuracy, precision, and robustness were considered following the ICH Q2 (R1) guidelines [12].

3.4.1. Specificity. Specificity is the ability to unequivocally assess an analyte in the presence of expected components [12]; in this case, the presence of IND-X and IND-Y in the indicator solutions and being responsible for the colour changes in the titrimetric analysis [1, 11]. In the test for specificity, five drops of 0.05% w/v ethanolic solutions of the test indicators were added to 10 mL of 0.1 M NaOH, 0.1 M HCl, and 0.1 M CH\(_3\)COOH in separate beakers, and the colour changes were noted. Titrations were then carried out using 0.1 M HCl and 0.1 M NaOH, respectively, and colour changes were again noted and recorded [1, 11]. Similarly, five drops of ethanol were also added to similar volumes of the abovementioned standard solutions, and similar investigations were carried out. Ethanol was observed not to have affected the original colours of the standard solutions. There were also no observable colour changes in the titrations of such solutions with their respective titrant solutions. However, in the case of IND-X and IND-Y, colour changes were observed when added to the standard solutions, and changes in these colours occurred upon their titrations. This showed that the IND-X and IND-Y compounds were responsible for the observed effects and as such were selective ad specific.

3.4.2. Accuracy. The accuracy of an analytical method is the closeness of the test results obtained to the mean or the theoretical true value [12]. In this study, the accuracy associated with the use of the indicators was defined as the closeness of their titrimetric endpoints with that of conventional indicators; and this was determined by comparing results obtained from the use of IND-X and IND-Y with that of phenolphthalein for titrations involving 0.1 M HCl/0.1 M NaOH, phenol red, and phenolphthalein for titrations involving 0.1 M CH\(_3\)COOH/0.1 M NaOH and phenolphthalein for the titrimetric assay of ibuprofen [1, 11].
**Figure 2:** The ionisation of IND-X and IND-Y in an alkaline medium (bathochromic shift with pink colour). (a) UV-Vis spectrum of IND-X. (b) UV-Vis spectrum of IND-Y.

**Figure 3:** UV-Vis spectra of synthesised indicators (a: IND-X; b: IND-Y) in ethanolic/0.5 M NaOH solution. (a) UV-Vis spectrum of IND-X. (b) UV-Vis spectrum of IND-Y.

| Compounds | Colour in alkaline medium | Colour in neutral medium | Colour in acidic medium |
|-----------|---------------------------|--------------------------|------------------------|
| IND-X     | Pink                      | Orange                   | Yellow                 |
| IND-Y     | Pink                      | Red                      | Yellow                 |

| Indicators | NaOH vs. HCl | NaOH vs. CH₃COOH | Assay of ibuprofen |
|------------|--------------|------------------|--------------------|
| Colour     | pH range     | Colour           | pH range           | Colour | pH range |
| Methyl red | Yellow to red| 4.40–6.20        | Yellow to red      | —      | —        |
| Methyl orange | Yellow to orange| 3.00–4.4        | Yellow to orange   | —      | —        |
| Phenolphthalein | Pink to colourless| 8–10             | Pink to colourless | Pink to colourless | —      |
| IND-X      | Yellow to pink| 8.02–10.20       | Pink to colourless | 8.02–10.20 | Pink to colourless | 8.02–10.20 |
| IND-Y      | Yellow to deep pink| 8.00–10.20     | Pink to colourless | 8.00–10.20 | Pink to colourless | 8.00–10.20 |

—: not applicable.
Figure 4: Various proposed forms of ibuprofen under acidic and alkaline conditions.

Figure 5: Accuracy investigations involving IND-X and IND-Y. (a) Comparing results from the use of IND-X with methyl orange (MTO) in HCl/NaOH titrations (n = 10); (b) comparing results from the use of IND-Y with MTO and methyl red (MTR) in HCl/NaOH titrations (n = 10); (c) comparing results from the use of IND-X with phenolphthalein (PHEN) and phenol red (PHR) in CH₃COOH/NaOH titrations (n = 10); (d) comparing results from the use of IND-Y with phenolphthalein (PHEN) in CH₃COOH/NaOH titrations (n = 10); (e) comparing results from the use of IND-X with phenolphthalein (PHEN) in the titrimetric assay of ibuprofen powder (IBP-PWD) and ibuprofen tablets (IBP-tab) (n = 10); (f) comparing results from the use of IND-Y with phenolphthalein (PHEN) in the titrimetric assay of ibuprofen powder.
Table 3: Results showing precision of outcomes from the use of the proposed indicators.

| Precision parameter | IND-X (mean ± SD; RSD) | IND-Y (mean ± SD; RSD) |
|---------------------|-------------------------|-------------------------|
|                     | HCl vs. NaOH | CH₃COOH vs. NaOH | Assay of ibuprofen | HCl vs. NaOH | CH₃COOH vs. NaOH | Assay of ibuprofen |
| Repeatability       |             |                  |                     |             |                  |                     |
| Different conc.     |             |                  |                     |             |                  |                     |
| 1. 0.05% w/v        | 10.01 ± 0.120; 1.20% | 9.88 ± 0.140; 1.42% | 99.66 ± 0.826; 0.83% | 10.69 ± 0.074; 0.69% | 10.49 ± 0.120; 1.14% | 101.0 ± 1.890; 1.87% |
| 2. 0.10% w/v        | 10.04 ± 0.158; 1.57% | 10.03 ± 0.082; 0.82% | 99.68 ± 0.554; 0.56% | 10.82 ± 0.045; 0.41% | 10.64 ± 0.089; 0.84% | 101.0 ± 1.446; 1.43% |
| 3. 0.15% w/v        | 10.02 ± 0.162; 1.62% | 9.80 ± 0.103; 1.04% | 99.32 ± 0.841; 0.85% | 10.64 ± 0.055; 0.51% | 10.50 ± 0.071; 0.67% | 101.0 ± 1.687; 1.67% |
| Intermediate        |             |                  |                     |             |                  |                     |
| precision           |             |                  |                     |             |                  |                     |
| Different days       |             |                  |                     |             |                  |                     |
| (1) Day 1           | 10.01 ± 0.120; 1.20% | 9.98 ± 0.092; 0.92% | 100.3 ± 0.585; 0.58% | 10.82 ± 0.045; 0.41% | 10.44 ± 0.055; 0.52% | 101.0 ± 0.477; 0.47% |
| (2) Day 2           | 9.78 ± 0.114; 1.16% | 10.08 ± 0.123; 1.22% | 100.1 ± 0.288; 0.29% | 10.84 ± 0.089; 0.83% | 10.52 ± 0.084; 0.80% | 100.2 ± 1.822; 1.82% |
| (3) Day 3           | 9.94 ± 0.178; 1.79% | 9.90 ± 0.125; 1.26% | 100.1 ± 0.187; 0.19% | 10.76 ± 0.114; 1.06% | 10.50 ± 0.123; 1.17% | 102.4 ± 1.243; 1.21% |
In 0.1 M HCl/0.1 M NaOH titrations, it was observed that the endpoints produced from the use of IND-X were comparable with that of methyl orange (t = 1.233, df = 18, p = 0.2336; Figure 5(a)). When IND-Y was used, the endpoints were also comparable with that of methyl orange and methyl red (F(2, 27) = 2.110, p = 0.1408; Figure 5(b)). In titrations involving 0.1 M CH₃COOH and 0.1 M NaOH, similar comparable endpoints were observed from the use of IND-X and phenol red and phenolphthalein (F(2, 27) = 2.057, p = 0.1474; Figure 5(c)), as well as that from the use of IND-Y and phenolphthalein (t = 0.8585, df = 18, p = 0.4073; Figure 5(d)). Also, in the titrimetric assay of ibuprofen, both indicators proved to produce similar results as phenolphthalein, which is the recommended indicator in the compendial method (Figures 5(e) and 5(f)) [13]. In all, the proposed indicators demonstrated comparable analytical qualities, in terms of the accuracy of their outcomes when compared to already established and widely used indicators.

3.4.3. Precision. Precision is defined as the closeness of agreement of results under defined conditions [12]. In testing for precision, both repeatability and intermediate precision were considered. For repeatability, replicate determinations of the endpoints with the use of 0.5% (w/v) concentrations of IND-X and IND-Y were carried out under the considered titrimetric applications. Titrations with different concentrations of the indicators were also considered. In each of the instances, the relative standard deviations calculated on the replicate endpoints and assays were compliant with the acceptance criteria of <2.00% (Table 3). In effect, the use of the two indicators resulted in precise results.

3.4.4. Robustness. In the test for robustness, different number of drops of the 0.05% (w/v) concentrations were considered in the titrimetric analysis, and these were 2, 3, and 4 drops. The results as shown in Table 4 indicate that irrespective of the number of drops used, and the endpoints in each titrimetric scenario were comparable for both indicators. Also, the RSDs determined were mostly compliant with the acceptance criteria of ≤2%, except for using 4 drops of IND-X in titrations involving NaOH and HCl (Table 4).

| Indicator | Type of titration | No. of drops | Mean ± SD, RSD | One-way ANOVA |
|-----------|------------------|--------------|---------------|---------------|
| IND-X     | NaOH vs. HCl     | 2 drops      | 10.02 ± 0.162, 1.62% | F(2, 27) = 1.105; p = 0.3456 |
|           |                  | 3 drops      | 10.01 ± 0.120, 1.20% |               |
|           |                  | 4 drops      | 9.92 ± 0.204, 2.06% |               |
|           |                  | 2 drops      | 9.91 ± 0.129, 1.30% |               |
| NaOH vs. CH₃COOH | 3 drops      | 9.90 ± 0.149, 1.51% |               |
|           |                  | 4 drops      | 9.98 ± 0.123, 1.23% |               |
| IND-Y     | NaOH vs. HCl     | 2 drops      | 11.19 ± 0.120, 1.07% | F(2, 27) = 2.241; p = 0.1258 |
|           |                  | 3 drops      | 11.16 ± 0.097, 0.87% |               |
|           |                  | 4 drops      | 11.10 ± 0.067, 0.60% |               |
| NaOH vs. CH₃COOH | 2 drops      | 10.49 ± 0.120, 1.14% |               |
|           |                  | 3 drops      | 10.47 ± 0.095, 0.91% |               |
|           |                  | 4 drops      | 10.50 ± 0.067, 0.63% |               |

4. Conclusion

The acid-base indicator property of IND-X and IND-Y have been evaluated and validated according to the ICH standards. Both indicators were found to serve as an alternative indicator to methyl orange, methyl red, phenol red, and phenolphthalein in titrations involving strong acids/strong bases and weak acids/strong bases. Also, both indicators have demonstrated suitable alternatives to phenolphthalein in the titrimetric assay of ibuprofen-related products. Hence, 4-(2-(2,4-dinitrophenyl) hydrazone) methyl)-2 methoxy phenol and 4-(2-(2,4-dinitrophenyl) hydrazone) methyl benzene-1,3-diol can be employed as suitable indicators for conventional titrimetric analysis in student demonstration laboratories and the quality control of ibuprofen-related products. In terms of sustainability, a starting material like vanillin for IND-X are readily available and can be obtained from a natural source without much cost compared to the standard indicators mentioned. In terms of precision, IND-X is generally the best for the assay of ibuprofen, whilst IND-X is more suitable for the NaOH/HCl and NaOH/CH₃COOH titrations.

Data Availability

Characterisation and analytical data are available at the Synthetic Chemistry Laboratory, Department of Pharmaceutical Chemistry, School of Pharmacy, University of Health and Allied Sciences, Ho.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

C. D. K. A, E. O, A. B, I. A, A. A developed the concept. C. D. K. A synthesised the compounds, and S. A., B. M. A and E. O carried out the titrimetry. C. N., D. A and J. S. A. carried out the spectroscopic analysis and pH determinations. C. D., K. A and E. O wrote the draft manuscript. A. B, I. A, and P. K. S reviewed and edited the draft manuscript.
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