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# Qualitative and quantitative estimation of nitrogen and oxygen anions: Utilization of No-D PULCON NMR

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This study introduces an analytical method designed for the comprehensive analysis of reaction intermediates, encompassing both qualitative identification and quantitative determination within reaction mixtures, with specific application to processes that generate anionic species. The approach synergistically integrates two NMR techniques No-D and PULCON to afford mechanistic insight into anion-formation pathways. The No-D NMR technique provides qualitative analysis of anion formation, while PULCON enables quantitative measurement of anion concentrations. The method has been validated and tested on 29 different reaction samples, yielding well-correlated results.

**Keywords:** $^{1}$H NMR, No-D NMR, PULCON, Anion quantification, Reaction monitoring

The optimization of chemical reactions is a critical step in organic synthesis to maximize the yield of target compounds[1,2]. Chemical reaction optimization typically involves systematic adjustment of key parameters such as concentration, temperature, *p*H, and reagent ratios. Throughout this process, it's crucial to regularly analyze the reaction profile by monitoring unreacted starting materials, tracking the formation of intermediates, and measuring product formation[3,4]. This ongoing analysis ensures that optimal amounts of reagents are used for reaction completion. By closely monitoring the progress, chemists can effectively understand their synthetic processes, leading to improved control and reproducibility of a chemical reaction. When the analysis of reaction intermediates presents significant challenges due to their inherent instability and high reactivity. These intermediates often rapidly undergo undesired chemical transformations, decompose during conventional analysis, or react with internal standards, making their monitoring particularly difficult. To obtain reliable results when examining such intermediates, researchers generally employ specialized techniques and conditions. These may include the use of inert atmospheres to prevent unwanted reactions with air or moisture, cryogenic conditions to slow down decomposition processes, and rapid analysis methods to capture data before the intermediates transform. Reactive intermediates are often stabilized through derivatization for quantification and reaction monitoring. However, this approach may yield inaccurate conclusions about reaction progress, as the derivatization process is frequently non-quantitative.

To address these limitations, we investigated the use of No-D NMR [5-9] and PULCON[10] techniques. No-D NMR has emerged as a powerful analytical technique in various fields of chemistry, gaining significant popularity in recent years due to its simplicity, precision, and cost-effectiveness. PULCON is an external standard technique for quantification in NMR spectroscopy[11-15]. This method correlates the absolute intensities of two one-dimensional (1D) NMR spectra to determine unknown concentrations[16-19]. Accurate quantification of unknown sample concentrations by PULCON requires that the external standard's concentration be known. This approach offers a robust and efficient means of quantitative analysis in NMR spectroscopy without the need for internal standards. Recently, Toshiyuki Suzuki and colleagues[20] have demonstrated the utility of No-D PULCON NMR in quantifying okadaic acid, a valuable marine toxin. In a separate study, Ghiviriga and co-workers explored the effectiveness of the technique for determining concentrations of commercial organometallic reagents. Their research revealed a high degree of concordance between PULCON results and those

obtained through conventional titration methods, with error margins falling within acceptable limits[21]. These studies collectively highlight the versatility and reliability of No-D PULCON NMR as a quantitative analytical tool across diverse chemical domains. To the best of our knowledge, there is no method exists for monitoring and quantification of oxygen and nitrogen anions during chemical processes by using No-D PULCON NMR. Herein, we report a convenient and versatile methodology for monitoring and quantitation of oxygen and nitrogen anions during chemical reactions.

## Results and Discussion

We began our investigation with aniline as a model substrate. Our primary aim was to perform a qualitative analysis of the progressive anion formation induced by base-mediated reactions, utilizing No-D NMR spectroscopy. The experiment involved the incremental addition of 1M of lithium hexamethyldisilazide (LiHMDS) in tetrahydrofuran (THF) in varying equivalents to a stirred solution of aniline in THF at ambient temperature. The generation of anion was subsequently monitored through No-D NMR techniques. In the first experiment, 0.3 equivalent of LiHMDS was added, and after 10 min of stirring at RT, an aliquot of the reaction mixture was taken in the NMR sample tube, and No-D $^1$H NMR data were recorded. The $^1$H NMR indicated that there is a change in chemical shift of $NH_2$ protons of aniline from δ 4.48 to 4.07 (Fig. 1), which indicated the formation of an anion. However, no separate chemical shift was observed for unreacted aniline and aniline anion lithium salt due to the equilibrium shown in Scheme 1.

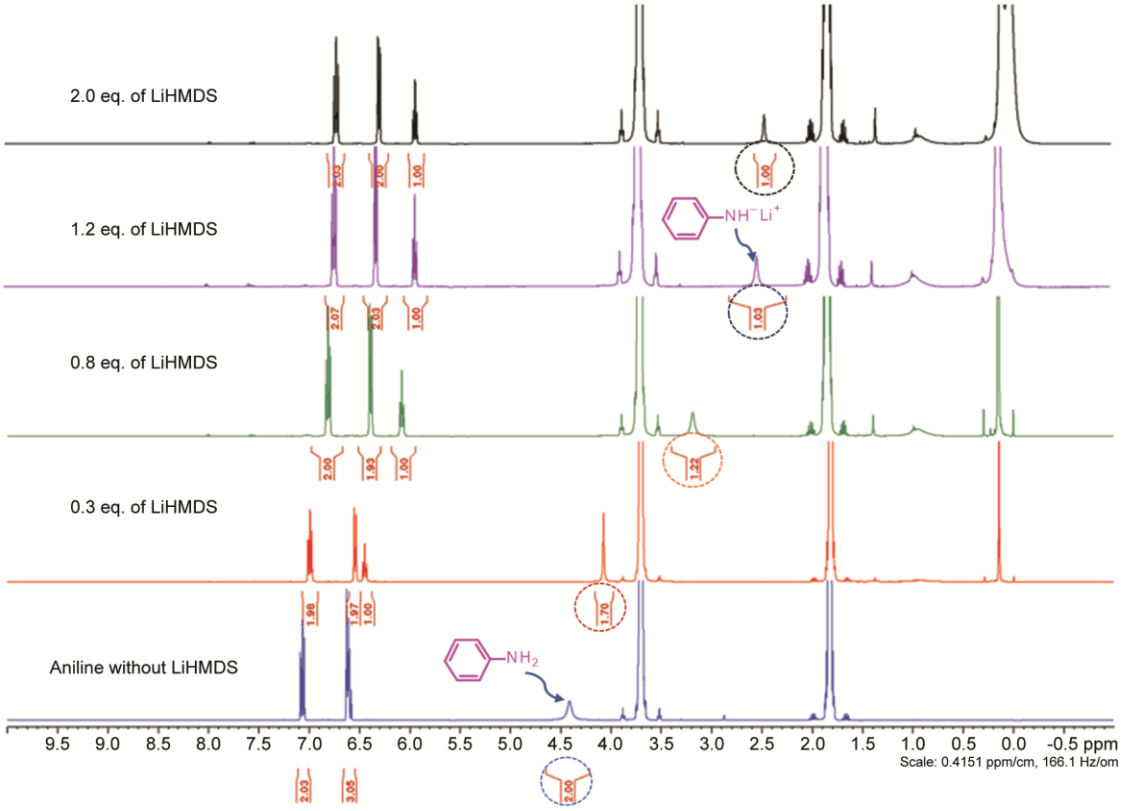
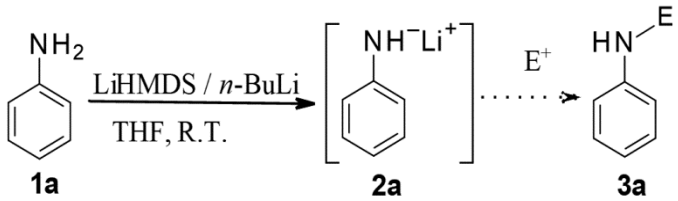



Fig. 1 — No-D $^1$H NMR spectrum of aniline and aniline anion generated with different equivalents of LiHMDS



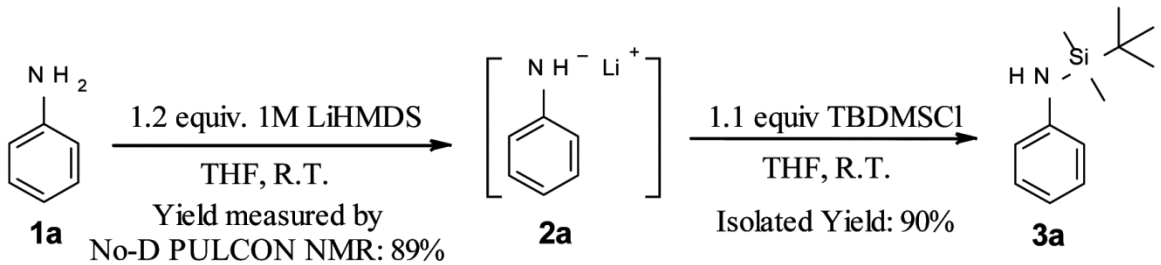

Scheme 1 — Study for the progress of aniline anion formation in THF with varying amounts/equivalents of LiHMDS and *n*-BuLi

The $NH_2$ proton was then integrated with a single aromatic proton of aniline, which indicated that 0.3 equivalent of integration is reduced. The reduction of $NH_2$ protons integration implies the formation of the nitrogen anion of aniline (Fig. 1). In the next experiment, 0.5 equivalent of LiHMDS was added. Like the first experiment, $^1$H NMR of the reaction aliquot was recorded, and integration of the $NH_2$ proton indicated formation of 0.48 equivalent of nitrogen anion. Similarly, four more experiments were performed with aniline by the addition of 0.8 equivalent, 1.0 equivalent, 1.2 equivalent, and 2.0 equivalents of LiHMDS solution. No-D NMR of the experiments with 0.8 equivalent and 1.0 equivalent of LiHMDS indicated formation of 0.78 equivalent and 0.98 equivalent of the nitrogen anion of aniline, respectively. However, no further anion formation was observed with excess amount (1.2 equivalents and 2.0 equivalents) of LiHMDS, as expected[22]. The overlayed NMR spectra of reaction aliquots from experiments with varying equivalents of LiHMDS offer a comprehensive visualization of the progressive formation of the aniline anion. These spectra effectively highlight the -$NH_2$ proton as a characteristic signal, providing valuable insight into the anion formation process. The clear spectral trends observed in this overlay (Fig. 1) corroborate our hypotheses regarding the reaction's dependence on the amount of base. Similar to LiHMDS study, quantification of aniline anion generated from aniline by using 2M of *n*-BuLi in cyclohexane was conducted by No-D NMR. Four different experiments were conducted with aniline in THF by addition of *n*-BuLi. The aniline anion formed with 0.3 equivalent, 0.5 equivalent, 0.8 equivalent, and 1.0 equivalent of *n*-BuLi were qualitatively monitored by No-D NMR and provided similar results as experiments with LiHMDS (Fig. S2). Figure S3 highlights a direct comparison of the reaction outcomes across different stoichiometric ratios of LiHMDS and *n*-BuLi.

Encouraged by the successful qualitative measurement of aniline anion formation using No-D NMR, we proceeded to employ PULCON for quantitative analysis of the generated anion. The method dispenses with the need for an internal standard and relies on the signal strength obtained with an NMR probe. Water was chosen as an external reference standard, and data acquisition and processing were done following the standard No-D NMR procedure. Results were quantified by using the PULCON relation, which is available in Bruker[23] Topspin under the name "Eretic2".

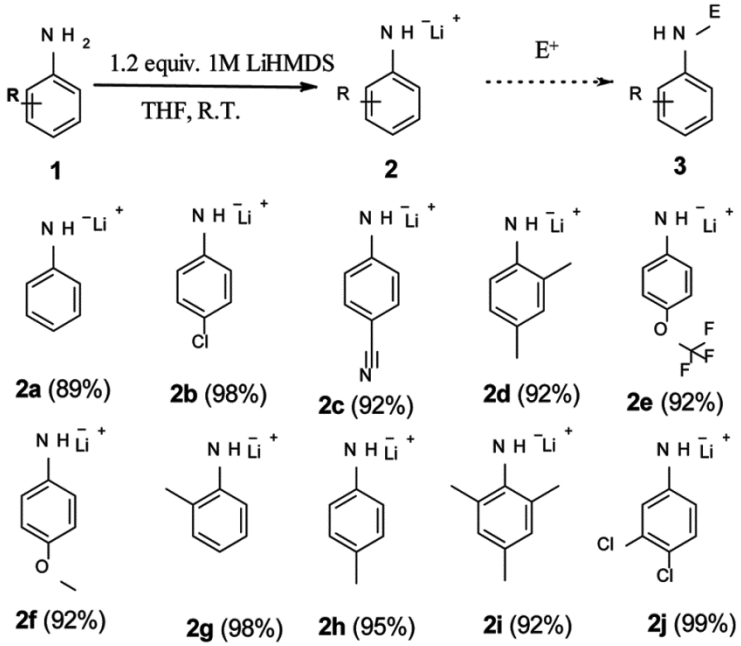
In the next experiment, we treated aniline with 1.2 equivalents of LiHMDS in THF at RT. No-D NMR of the reaction aliquot indicated complete disappearance of one $NH_2$ proton as before. PULCON experiment of the reaction aliquot was conducted concerning water as an external standard. The triplicated PULCON experiment indicated 89% formation of the aniline anion. To validate the methodology, our process of quantification, further, the reaction mixture was treated with 1.1 equivalent of TBDMSCl (tert-butyldimethylsilyl chloride)[24], which afforded aniline silyl derivation **3a** in 90% isolated yield (Scheme 2). Notably, this yield closely correlates with the anion formation yield measured by the PULCON NMR experiment. This correlation unambiguously confirms the reliability of the PULCON NMR method for quantifying the aniline anion.

A series of differently substituted anilines was tested for anion generation by using 1.2 equivalents of LiHMDS. All the experiments were conducted on 500 mg of starting aniline. All the anilines screened provided excellent conversion, and yields were successfully determined by No-D NMR PULCON. There was no significant difference observed for anilines bearing electron electron-donating group or electron electron-withdrawing group and sterically hindered anilines, probably due to the use of a strong base, LiHMDS (Table 1).




Scheme 2 — Comparison of the yield of aniline anion as lithium salt, measured by No-D PULCON NMR, and isolated yield of silyl derivative from aniline anion

Table 1 — Yields of anilines anion as lithium salts measured through No-D-PULCON NMR analysis




Next, we explored the feasibility of quantification study for indoles anion and phenols anion generated from corresponding indoles and phenols with 1.2 equivalent of LiHMDS in THF. Similar to the aniline anion quantification study, sampling for each experiment was conducted after 10 min followed by No-D PULCON NMR experiment. The formation of indoles anion and phenols anion was indicated by the disappearance of -NH proton and -OH proton at the same time, change in chemical shift of the ring protons was also observed (Fig. 2).

All the indoles and anilines subjected for experiments provided corresponding anion as lithium salt in excellent yields (Table 2). Like aniline, no significant yield difference was observed for different substitutions at aromatic ring of indoles (Table 2, **5a-j**) or phenols (Table 3, **7a-h**). We conducted the experiment with 5-methoxy indole. The yield of the anion generated from 5-methoxy indole with 1.2 equivalent of LiHMDS measured by PULCON NMR was 96.60% and isolated yield for corresponding silyl derivate[25] **6h** was 96.44% (Scheme 3). Both the experiments further confirmed the accuracy of PULCON experiment for measurement of strength of oxygen and nitrogen anion in reaction mixture.

We also tested the feasibility of quantification regioselective anion formation from amino phenols (Table 2, **7f-h**). As -OH in amino phenol is more acidic than -$NH_2$, it is expected to form phenolate first in the presence of strong base like LiHMDS. To our delight, when 2-amino-5-nitrophenol was treated with 1.0 equiv. LiHMDS in THF, corresponding phenolate was formed. The spectral data revealed several significant observations like the signal previously observed at δ 9.08 attributed to the hydroxyl proton (-OH), which was no longer detectable in the spectrum of the reaction mixture. This suggests complete deprotonation of the phenolic group the resonance corresponding to the amino protons (-$NH_2$) exhibited a slight up field shift from δ 5.62 to 5.43. The yield of phenolate obtained from 2-amino-5-nitrophenol was determined to be 94.54% using the PULCON NMR experiment. The reaction mixture was then treated with 1.2 equivalent of TBDMSCl to provide corresponding O-silyl product **8g** in 92.86% chemical yield (Scheme 4), the structure of **8g** was unambiguously confirmed by 2D NMR and other means of analytical tools.

The additional investigation involved a revalidation of the results using several external standards, namely 1,5-cyclooctadiene (1,5 COD), 1,3,5-trimethoxybenzene (1,3,5 TMB), and 1,2,4,5-tetramethylbenzene (1,2,4,5 TB). The use of multiple external standards allows for a comprehensive and comparative analysis, enabling a more thorough understanding of the composition of the reaction mass. We performed three separate samplings from 10 different reaction mixtures and determined the

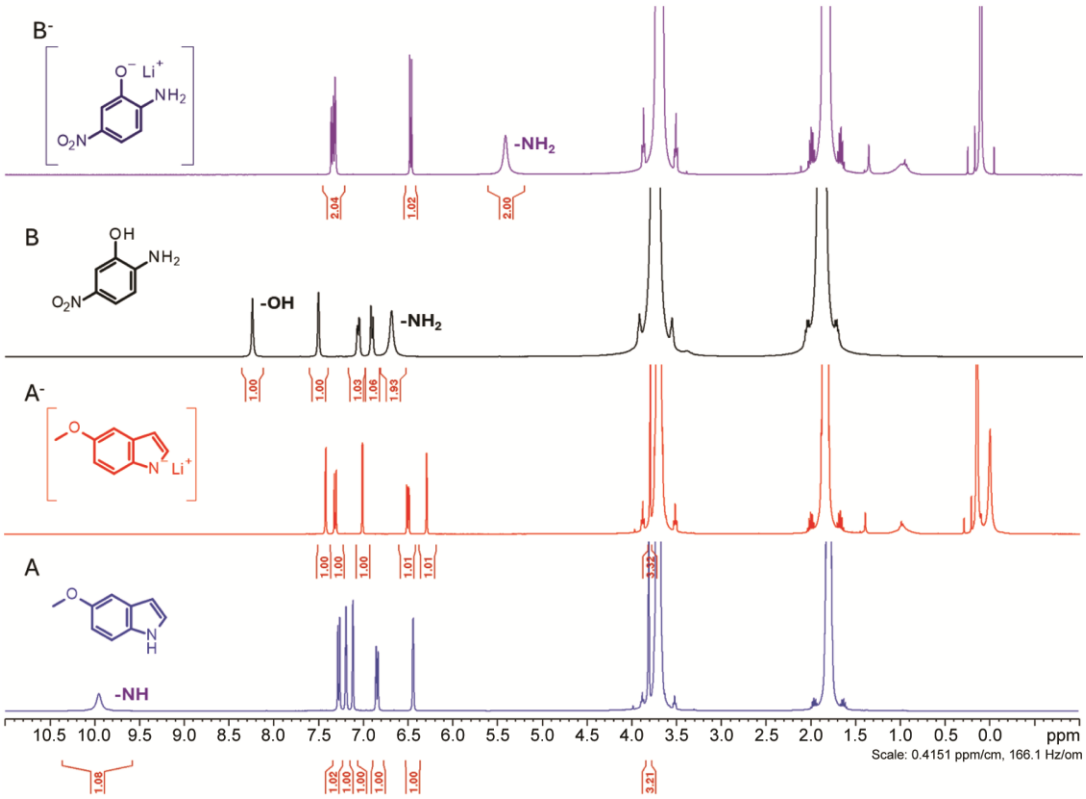



Fig. 2 — No-D $^{1}$H NMR spectrum shows abstraction of -NH and -OH proton from indole and phenol respectively by using base LiHMDS

Table 2 — Yields of indoles and phenol anions as a lithium salt measured through No-D-PULCON NMR analysis

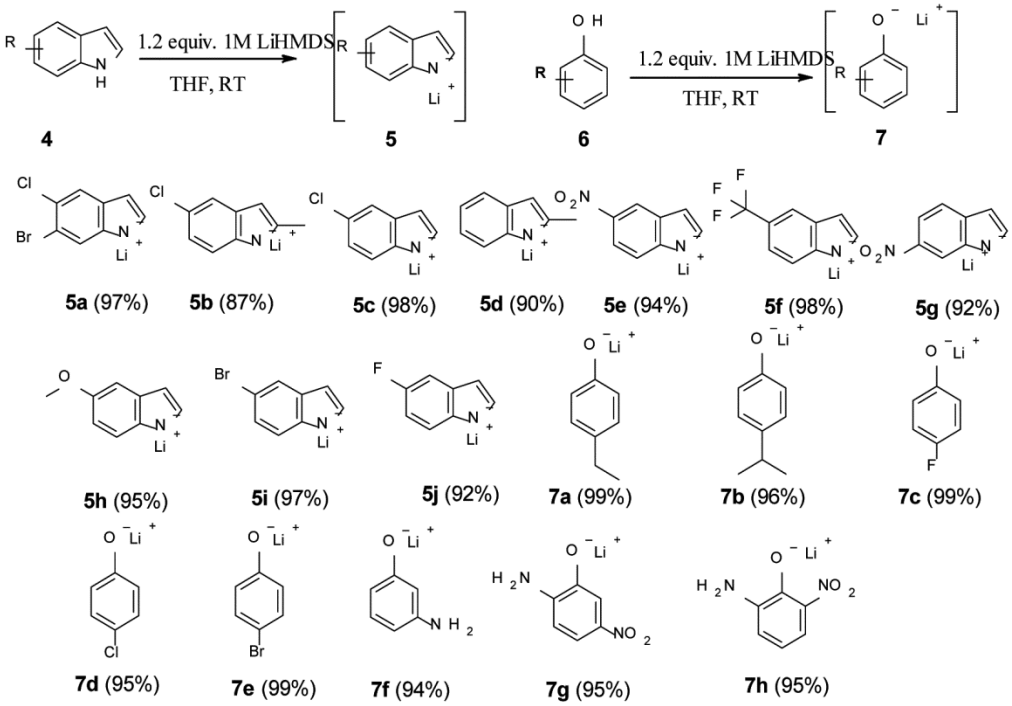
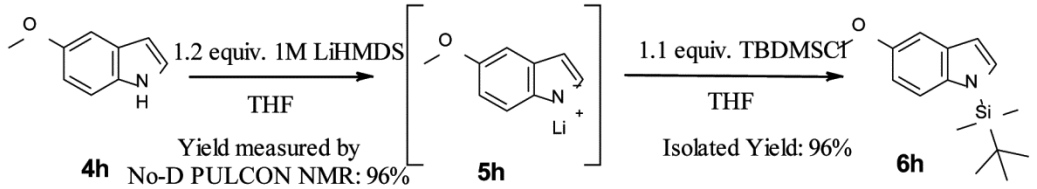
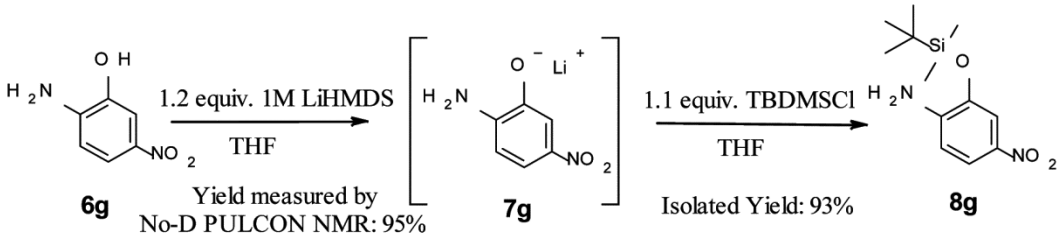


Table 3 — Anion concentrations result determined by using four different external standards water, 1,5 COD, 1,3,5 TMB, and 1,2,4,5 TB

| Name of Compd | Measured concentration of anions (%) by using external standards | | | | Avg | Std. Dev | RSD |
|---|---|---|---|---|---|---|---|
| | Water | 1,3,5 TMB | 1,2,4,5 TB | 1,5 COD | | | |
| 2,4-Dimethylaniline | 91.53 | 92.19 | 91.4 | 91.75 | 91.72 | 0.35 | 0.38 |
| o-Toluidine | 98.49 | 99.23 | 98.35 | 98.73 | 98.70 | 0.39 | 0.39 |
| 2,4,6-Trimethylaniline | 92.5 | 93.19 | 92.38 | 92.73 | 92.70 | 0.36 | 0.39 |
| 3,4-Dichloroaniline | 98.6 | 99.33 | 98.28 | 98.85 | 98.77 | 0.44 | 0.45 |
| 6-Bromo 5-chloroindole | 96.87 | 97.57 | 96.74 | 97.1 | 97.07 | 0.37 | 0.38 |
| 5-Chloroindole | 98.3 | 98.96 | 98.15 | 98.52 | 98.48 | 0.35 | 0.36 |
| 5-Trifluromethylindole | 98.04 | 98.75 | 97.91 | 98.28 | 98.25 | 0.37 | 0.38 |
| 5-Methoxyindole | 94.72 | 95.41 | 93.77 | 94.96 | 94.72 | 0.69 | 0.73 |
| 4-Flurophenol | 98.85 | 99.57 | 98.75 | 99.09 | 99.07 | 0.37 | 0.37 |
| 4-Chlorophenol | 95.46 | 96.41 | 95.18 | 95.95 | 95.75 | 0.54 | 0.57 |




Scheme 3 — Comparison of yield of indole anion as lithium salt, measured by No-D PULCON NMR and isolated yield of silyl derivative from aniline anion




Scheme 4 — Comparison of yield of phenol anion as lithium salt, measured by No-D PULCON NMR and isolated yield of silyl derivative from aniline anion

composition using four different external standards. Upon comparing these results with all the standards, we found the outcome is a high degree of concordance, with variations among the standards being 2% (Table 3).

Overall, our findings suggest that this method used for quantification of the anion is robust, precise, and capable of producing highly consistent results. This level of concordance and minimal variation among the standards underscores the reliability and accuracy of the analytical approach, which is essential for drawing meaningful conclusions about the quantification of the anion in reaction mass.

## Materials and Methods

### Materials

We obtained all external standards from Sigma Aldrich, including 1,5 Cyclooctadiene, 1,3,5 Trimethoxy benzene, and 1,2,4,5-Tetramethyl benzene. LiHMDS (Sigma Aldrich, 1M in THF) and THF (Thermo Fisher Sci.) were also sourced from these suppliers. Additionally, the other chemicals used as starting materials for this study were purchased from Aldrich Chemical Co., Thermo Fisher Sci., Chempure, Tokyo Chemical Industry, Alfa Aesar Chemicals, and Avra Synthesis Private Limited, and were used as received.

### Instrumentation

The NMR spectra were acquired using a BRUKER Avance III HD 400 MHz NMR spectrometer with a 5 mm probe head (PA BBO 400S1 BBF-H-D-05 Z) operating at 400.2 MHz for $^{1}$H NMR. Subsequently, the NMR spectra were processed using BRUKER TOPSPIN 3.6.3. The essential acquisition parameters can be found in the supplementary information

(**Table S2**). All samples were measured in 5 mm NMR sample tubes which is Wilmad OD = 5mm length = 7".

Before starting the study, we need to obtain an external reference standards sample and then determine its concentration (mmol/l) in the Eretic tool. Eretic tool (based on PULCON) which is given an all-BRUKER TOPSPIN software. Using Eretic in the spectrum of our target analyte sample, we should select the "calculate concentration" option, where we define the number of protons responsible for a particular resonance or signal. By specifying the protons, we can obtain the concentration of our analyte. NMR data acquisition and processing parameters should be same for both analyte and standard like number of scans (ns), delay (d1) and pulse angle, *etc*. (all details are given in supporting information) When samples are obtained without deuterated solvent; shimming, ensuring proper phase correction is crucial, as it can have a significant impact on the results. With the combination of PULCON and No-D NMR, we have assessed anion quantification of 29 compound. We employed 1.2 equivalent of LiHMDS (1.0 M) in THF to generate anions for all compounds. To a stirred solution of interested compounds in THF (2.5 mL) was added LiHMDS. After the addition of LiHMDS, an aliquot of reaction mixture was taken under inert/nitrogen condition in the NMR tube and $^{1}$H NMR data was recorded. In the $^{1}$H NMR spectrum, the characteristic signal corresponding to the α-methylene protons ($OCH_2$) of THF was referenced to δ 3.70 This resonance serves as an internal reference for spectral analysis and compound characterization.

## External reference standard preparations

The specific details of the external standards used are as follows: The external standards were individually prepared in 10.0 mL volumetric flasks, dissolved in THF. The quantities used for each standard were 1.2553 g of 1,2,4,5-tetramethylbenzene, 1.1702 g of 1,3,5-trimethoxybenzene, and 1.4952 g of 1,5-cyclooctadiene. The concentration of the external reference standards was determined to be 935.25 mmol/L for 1,2,4,5-tetramethylbenzene, 695.76mmol/l for 1,3,5-trimethoxybenzene, and 1382.14 mmol/L for 1,5-cyclooctadiene.

## Conclusion

Through No-D PULCON NMR techniques, an approach has been established for precise monitoring of reaction progress and quantification of nitrogen and oxygen anions of anilines, indoles and phenols. It was successfully applied to 29 diverse samples, showcasing its versatility in both monitoring and quantification processes. Key advantages of our approach include rapid analysis time, no requirement for additional specialized equipment, comprehensive reaction profiling within a single analytical technique. Its efficiency and adaptability make it particularly suitable for process optimization in synthetic chemistry. The method provides a powerful tool for researchers and chemists to gain deeper insight into reaction dynamics and mechanisms, potentially accelerating the development and optimization of chemical processes involving these important classes of compounds. Ongoing work in our laboratory is focused on extending this approach to the monitoring and quantification of diverse carbanions intermediates.

## Supplementary Information

Supplementary information is available in the website at https://nopr.niscpr.res.in/handle/123456789/5877.

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