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Review Article

4-CHLORO-7-NITROBENZOFURAZAN (NBD-CL) AS PRE AND POST-COLUMN DERIVATIZATION REAGENT FOR AMINE GROUPS ANALYSIS USING CHROMATOGRAPHY: A MINI-REVIEW

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ABSTRACT

Derivatization is a modification process to produce a new compound that can be detected using the suitable detector in chromatography analysis. Derivatization reagent is needed to chemically modify a compound. One of a derivatization reagent that is widely used as a fluorogenic and chromogenic reagent in amine group's analysis using chromatography is 4-Chloro-7-nitrobenzofurazan (NBD-Cl). NBD-Cl is considerable attraction because it plays a role in pre and post-column derivatizations fused in chromatography for increasing the selectivity and sensitivity. This review provides an overview of many papers that have been presented regarding the application of 4-Chloro-7-nitrobenzofurazan (NBD-Cl) in pre and post-column derivatization in amine group's analysis and its chromatographic condition from the literature between 2000 to 2022.

Keywords: 4-Chloro-7-nitrobenzofurazan, Amine groups, Chromatography, Pre column derivatization, Post column derivatization

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INTRODUCTION

Chromatography is the most frequently used technique for the determination of organic subtances in various matrices [1]. Analysis of several compounds require a derivatization step to increase the detection limits. Several derivatization reagents for amine groups such as ninhydrin [2], 2,4-dinitro-1-fluorobenzene [3], o-phthalaldehyde (OPA) [4], 1,2-naphtoquinone-4-sulfonate (NQS) [5], Sanger regent (FNDB) [6], fluorescamine [7], 5-(dimethylamino)naphthalene-1-sulfonyl chloride (Dansyl Cl) [8], and 4-Chloro-7-nitrobenzofurazan (NBD-Cl) [9]. NBD-Cl is quite popular because it has high sensitivity and can be used when analyzing low-concentration samples. It also low cost reagent with reasonable stability before analysis [10, 11].

NBD-Cl was synthesized by nitrating 4-chlorobenzofurazan, obtained from 2,6-dichloroaniline via the dichloronitrosobenzene [12]. The first application of NBD Cl as fluorogenic reagent was reported by Ghosh and Whitehouse when detecting glycine on thin layer chromatography plates with a Chromatavue long-wavelenth ultraviolet lamp [13]. NBD-Cl is more stable to moisture and more soluble in aqueous solutions than Dansyl Cl [14]. NBD-Cl is widely used as a fluorogenic reagent for the analysis of primary or secondary amines [15]–[20], amino acid [13], thiols [21], and lipids [22]. The chemical structure of NBD-Cl is given in fig. 1.

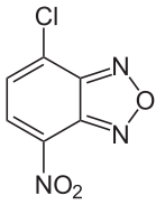

Fig. 1: Structure of NBD-Cl

Application of NBD-Cl as Pre-column derivative reagent

In pre-column derivatization, the reaction is performed before chromatography injection. The critical parameters, such as reaction time, solvent reaction, temperature, pH, must be selected and optimized [23]. The pre-column derivatization using NBD-Cl as a reagent for the analysis of amine groups is describe below.

The analysis of glyphosate herbicide and (aminomethyl) phosphonic acid (AMPA) in water using NBD-Cl was developed by Colin *et al.* [24]. The reaction was done at 50 °C at pH of 8.0. Two protocols were used, which were using C-18 column and the amino column. The limit of quantification was 1 µg/l for glyphosate and 0.1 µg/l for AMPA.

Tatar and Atmaca developed the method for the analysis of amlodipine. Solid phase column using silica column was used to extract amlodipine from plasma. The assay was linear over the concentration range 0.25 0 18.00 ng/ml [25]. A fast, simple, and specific HPLC method for the determination of amlodipine in human serum using propranolol as an internal standard was developed by Bahrami and Mirzaeei. Amlodipine was extracted by ethyl acetate. The limit of quantification was 0.25 ng/ml of serum [26]. Saputri *et al.* developed a sensitive bioanalytical method for the simultaneous determination of amlodipine and glibenclamide. 0.08% of NBD-Cl was used as derivatization reagent. The reaction was at pH 8.6 with Teorell and Stenhagen buffer at 70 °C for 20 min. 0.1 N sulfuric acid was used as stopper of the reaction [27].

Specific method for the determination of lisinopril in dosage forms with HPLC-fluorimetric detection was developed by Emam *et al.* [28]. This method based on the derivatization of lisinopril with NBD-Cl. Bumetanide was used as internal standard on a reversed phase ODS column using isocratic elution. Methanol and 0.02 M sodium dihydrogen phosphate at pH 3 (55:45) was used as mobile phase at flow rate 1 ml/min.

Bahrami and Mohammadi [29] develop a new, very sensitive, and simple method for the quantification of gabapentin in human serum. Protein precipitation by acetonitrile was used to extract gabapentin from human serum. Separation was performed using C18 column. Isocratic mode of mobile phase was used as a chromatographic system. The limit of quantification was 0,002 µ/ml. the method was applied for the bioequivalence study of two gabapentin preparation in 24 healthy volunteers.

A selective, sensitive, and precise HPLC method for the determination of fluoxetine and norfluoxetine in human plasma has been developed by Erturk *et al.* [30]. Extraction with n-hexane, followed by derivatization with NBD-Cl under weakly alkaline condition. NBD derivatives were extracted with chloroform after

acidification. The lower limits of quantification were 1 ng/ml and 0.1 ng/ml for fluoxetine and norfluoxetine, respectively.

A simple HPLC method was developed by Yang *et al.* [31] for the analysis of free amino acids in islets Langerhans. Separation was done using C18 column with acetonitrile and acetate buffer as mobile phase. Good linearity was obtained over a wide range of 0.42–42.11 μM for most amino acids. The limit of detection was within the range of 6.1–51 nM.

Farshchi *et al.* [32] developed the method for the determination of sertraline in serum using NBD-CL as a derivatization agent. Azithromycin was used as an internal standard. Extraction was done using the liquid-liquid extraction method. Reaction between sertraline and NBD-Cl was done at pH 9. Isocratic reversed phase was used as the chromatographic system. Detection and quantification limits were 0.5 and 2 ng/ml.

A novel micellar electrokinetic chromatographic method with laser-induced fluorescence detection for the determination of muscle relaxant drug baclofen (BAL) was developed by Wang *et al.* [33]. NBD-Cl was used to derivatize BAL and gabapentin (internal standard). Detection limit and quantification limit were 0.9 μg/l and 6.25 μg/l, respectively.

A novel pre-column derivatization for the determination of bupropion in pharmaceutical preparation, human plasma, and human urine using NBD-Cl to produce a fluorescent derivative has been described by Ulu and Tuncel [34]. Mexiletine was used as internal standard. Reversed-phase HPLC method with isocratic mode of mobile phase was used. Detection limits for bupropion in plasma and urine were 5 ng/ml and 10 ng/ml. The method gives good performance in validation parameters.

A simple and sensitive HPLC method for the analysis of topiramate in plasma using NBD-Cl was developed and validated by Milosheska *et al.* [35]. Bendroflumethiazide was used as internal standard. Analyte was extracted using dichloromethane. Quantification limit was 0.01 μg/ml.

A precise and sensitive method for the determination of nilotinib in spiked plasma, urine, and pharmaceutical capsule formulation was developed by Yilmaz *et al.* [36]. NBD-Cl was used as fluorotag in the borax buffer (pH 9). Chloroform gives the best recovery for the extraction of nilotinib in plasma. The method consists of reversed-phase HPLC and isocratic mode of mobile phase. The linear range wer 100-600 ng/ml in standard solution, plasma, and urine. The method validation was performed with good recovery, linearity, stability, accuracy, and presicion.

Bagary *et al.* [37] developed novel method for the determination of varenicline tartrate based on precolumn derivatization using NBD Cl using fluorescence detector. Isocratic elution was applied using methanol and distilled water as mobile phase. Separation was done using the reversed-phase HPLC method. Quantification limit for the determination of varenicline tartrate was 0.2 μg/ml.

Omer *et al.* [38] developed and validated a simple and efficient HPLC method for the determination of taurine in energy drinks using HPLC with photodiode array (PDA) and fluorescence (FLD) detection. The method is based on the derivatization of taurine with NBD-Cl at alkaline condition. The detection was at 472 nm for HPLC-PDA and 472 nm/530 nm for HPLC-FLD. The methods meet the requirement of validation. Detection limits were 0.296 mg/l for HPLC-PDA and 0.616 x 10^{-3} for HPLC-FLD.

A method of detecting dimethylamine (DMA) and diethylamine (DEA) using NBD-Cl was developed by Gao *et al.* [39]. NBD-Cl was used to the selective detection of secondary amines, including DMA and DEA. Detection limit was 0.1 mmol/l and 10 nmol/l for DMA and DEA.

From the presented papers, it is known that NBD-Cl is widely used for pre-column derivatization of primary and secondary amines. In pre-column derivatization, the system consumes less reagent and simple instrument configuration. It also offers good sensitivity.

Application of NBD-Cl as post-column derivative reagent

Post-column derivatization (PCD) is a one of chromatography method applied for increasing the sensitivity of detector. In PCD the reaction occur by adding a derivatization reagent after separation and before detection [40]. The application of NBD-Cl in PCD was carried out by Rigas *et al*. that used NBD-Cl for the determination of domoic acid in mussels by HPLC and fluorescence detector. The NBD-Cl reacts with domoic acid to produce a fluorescent product that has a peak at 469 nm (excitation) and 529 nm (emission). In order to reduce the background fluorescence that appear when the first reaction produce hydrolyzed product 4-hydroxy-7-nitro-benzo-2, 1,3-oxadiazole (NBD-OH), they used hydrochloric acid as a second reagent. This system was running by RP-HPLC using nucleosil C18 column with 87% H2O with 0.1% trifluoroacetic acid (TFA) and 13% CH3CN as mobile phase isocratically. The result show that this method offer fully automated analysis, clean chromatogram, low detection limit in 25 ppb in real sample of muscle extract, and the major advantage of this method is absence of the fluorescence of the product of the NBD-Cl with tryptophan [41].

Other research of Rigas et. al. was using NBD-Cl as PCD for primary and secondary amines analysis by ion exchange chromatography. Five primary (methylamine, isoamylamine, 2-phenylethylamine, putrescine, and histamine) and one secondary amine (dimethylamine) were separated isocratically on a cation exchange column using HNO3 ($5x10^{-3}$ mol/L) as the mobile phase. This system offer low detection limit on 20-100 μgl^{-1} [42].

PCD offer several advantages compared to pre-column derivatization. PCD require less sample pretreatment and clean up before HPLC separation, less interferences from the reagent, the labeling can be automated and reproducible [41]. However, PCD requires fast kinetics of the labeling reaction and high sensitivity of the PCD reagent towards the analytes that some of the reaction cannot be applied in PCD analysis [43]. Other disadvantage of this system is the additional equipment may be required to heat and mix the eluent chromatography with the derivatization reagent to provide sufficient time for the reaction [43].

CONCLUSION

Pre and post-column derivatizations are the column techniques fused in chromatography for increasing the analytical performance. NBD-Cl is derivatization reagent that widely used in pre and post-column derivatization for amine groups analysis. NBD-Cl in pre-column derivatization plays a role as modifying the reagent before injected in chromatography system so it doesn't need additional equipment. Meanwhile in post-column derivatization, NBD-Cl is reacted after the sample was separated in column so the analysis will not be affected by the matrix sample. The use of NBD-Cl as a pre column derivatization reagent is more widely than post-column derivatization reagent due to simpler in instrument configuration. NBD-Cl can be applied for pre or post-column derivatization and both of the methods result good selectivity and sensitivity.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

Declared none

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