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Novel ecofriendly spectrophotometric methods for the determination of six dihydropyridines calcium channel blockers through derivatization with sulfophtalein dye: application to tablet analysis

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Abstract

Novel, «green» and simple visible spectrophotometric procedures for the determination of six dihydropyridines CCBs (amlodipine besylate (AML), lacidipine (LAC), levamlodipine besylate (LAML), nifedipine (NIF), nimodipine (NIM) and nitrendipine (NIT)) through derivatization with the sulfophthalein dye bromophenol blue (BPB) have been developed. The optimal parameters for CCBs spectrophotometric analysis via complex formation using BPB were as follows: detection wavelength—596 nm, reaction time—5 min, ratio of reacting components—1:1, operating temperature—25 ± 2 °C. The concentration was linearly proportional to absorbance values in the range of 3.40—17.00 µg/mL (AML), 1.14—9.11 µg/mL (LAC), 1.14—9.08 µg/mL (LAML), 4.16—12.40 µg/mL (NIF), 0.84—5.86 µg/mL (NIM), 6.52—19.60 µg/mL (NIT). The developed methods are colorimetric and therefore does not require a UV instrument to quantify these drugs. The proposed approach was more efficient in terms of time reliability, sensitivity and «greenness» than other recorded spectrophotometric methods and can be easily implemented for routine pharmaceutical analysis.

Keywords Calcium channel blockers, Bromophenol blue, Spectrophotometry, Assay, Validation

Introduction

According to the last data analysis of the World Health Organization, hypertension has a leading position in cases causing death. Approximately 1.35 billion of individuals in the world suffer from hypertension [1]. Various antihypertension classes, including diuretics, α- and β-blockers, angiotensin converting enzyme (ACE) inhibitors, and calcium channel blockers (CCB), are widely prescribed all over the world. Plenty of people with high blood pressure (HBP) require treatment that is absolutely available, safe, and effective for their needs [2]. CCB are a category of medicines mostly used to treat hypertension (Fig. 1). These drugs are also helpful in other pathological states. CCBs are classified into two classes based on their formula and duration: dihydropyridine and non-dihydropyridine. Besides that, dihydropyridines exist in four generations [3–16].

High-performance liquid chromatography (HPLC) and redox titration methods have been presented in the monographs of Pharmacopeias for the assay of various CCBs in bulk and dosage form [17, 18]. Plenty of available literature reviews have been revealed regarding

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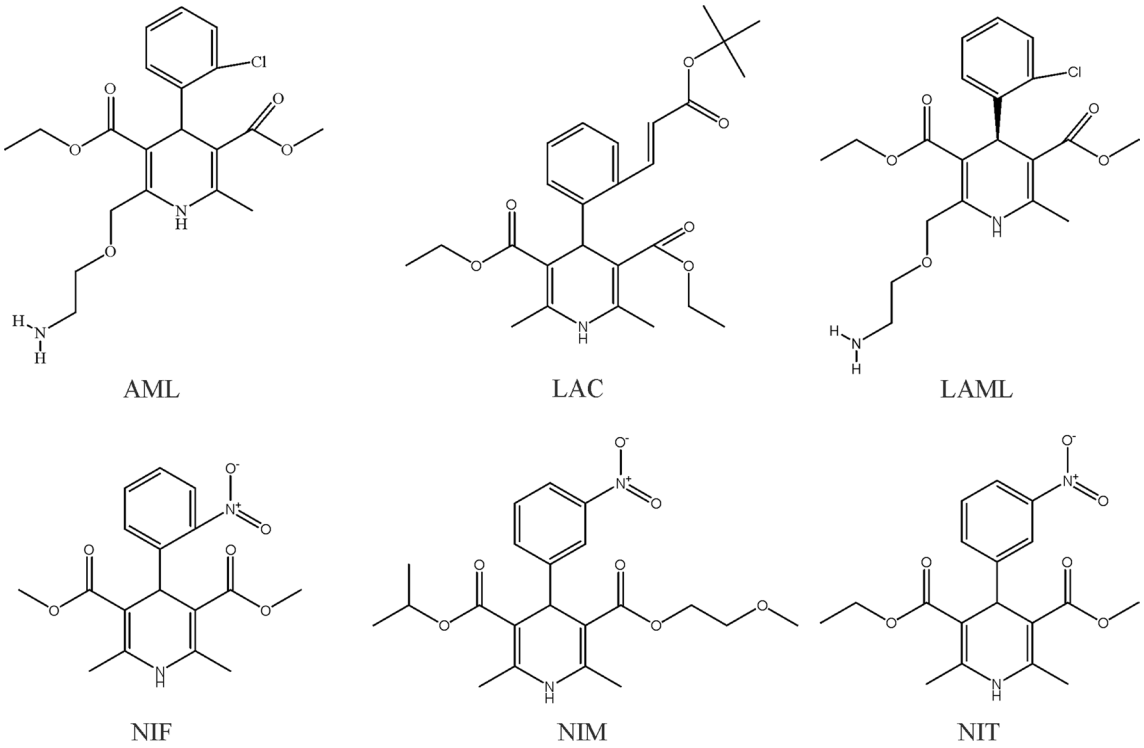


Fig. 1 Chemical structure of the discussed dihydropyridine CCBs. Amlodipine (AML), Lacidipine (LAC), Levamlodipine (LAML), Nifedipine (NIF), Nimodipine (NIM), Nitrendipine (NIT)

chromatographic and tandem spectrometry procedures, for instance, HPLC or reverse phase HPLC determination of CCBs in tablets and human plasma [19, 20]. Liquid chromatography mass spectrometry procedures for bioavailability and chiral conversion were preferred [21, 22]. Alternative approaches, including rapid capillary electrophoresis for the determination of enantiomeric contaminants [23], molecular docking [24] and investigation of binding characteristics, were described [25]. Even though these approaches are extremely helpful, specific, and sensitive, most of them were require critical conditions such as costly, time-consuming, laborious, and professional operation. Considering the simplicity, cheapness, low reagent consumption, availability in the most quality control laboratories and greenness of spectrophotometric techniques, quite a few procedures have been highlighted in the literature sources for the analysis of CCBs in bulk, pharmaceutical forms and biological fluids [26, 27]. A number of UV-spectrometric measurements have been performed in different polar and non-polar solvents for the discussed CCBs [28–31]. Micelle enhanced spectrofluorimetric determination of some CCBs was also reported [32]. For increasing specificity, some authors proposed interactions between the analyzed CCBs and some reagents, which are presented in Table 1. Most of the presented procedures have limitation (involve reducing of the analyzed CCBs, heating, damaging reagents, or acidic or alkaline mediums) that are negative for modern application of technique. Sulfophthalein dyes are commonly used as indicators in acid–base titrations. It was considered that these molecules colored and absorbed visible light. According to the authors [51], dyes were defined as old, but gold. For the spectrophotometric determination some CCBs, varieties of dyes have been used, such as bromophenol blue (BPB), bromothymol blue (BTB), bromocresol green (BCG) etc. It is worth noting, that proposed procedures contain pros and cons, which are illustrated in Table 2. The most commonly analyzed spectrophotometric procedures for assay CCBs with dyes had a lack of disadvantages that included extraction, pH adjustment, non-ecofriendly reagents, etc.

The present paper represents a novel, «green» and simple visible spectrophotometric procedure for the determination of six dihydropyridines CCBs based on their interaction with the sulfophthalein dye bromophenol blue (BPB).

Experimental

Instrumentation

As analytical devices Shimadzu UV-1800 double beam UV–vis spectrophotometer (Japan) with attached UV-Probe version 2.70 software was employed to perform

Table 1 Spectrophotometric determination of some CCBs with utilized reagents

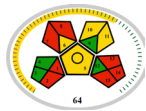
№	Dihydropy-ridine CCBs and sample	Utilized reagents	Conditions	Linearity, µg/mL	Limitation	MOGAPI score	Reference
1	AML, dosage forms	p-Chlor-anilic acid	Intermediate molecular association complex measured at 540 nm	100.0–600.0	1,4-dioxan Chloroform		[33]Rahman N., 2000
2	AML, drug formulation	Ninhydrin	Colored complex measured at 595 nm	10.0–60.0	DMF, heating		[34]Rahman, N., 2001
3	LAC, tablets	Ferric chloride, potassium ferricyanide	Bluish green colored chromogen measured at 740 nm	0.0–10.0	HCl		[35]Ravi-chandran Veerasamy, 2004
4	AML, pharmaceutical formulation	Ferric-1,10-phenan-throline	Colored complex measured at 510 nm	2.0–10.0	sodium acetate–acetic acid buffer		[36]Rahman N., 2004
5	NIF NIM AML, tablets	KMnO4 and cerium (IV) ammonium sulfate	Decrease the color of KMnO4 measured at 525 nm Fluorescence measured at 355 nm	5.0–30.0 0.2–1.0	H_2SO_4 Chloroform acidic KMnO4		[37]Askal H. F., 2010
6	pure and in pharmaceutical preparations	Chromo-tropic acid	Colored azo-dye measured at 520 nm	0.0–35.0	HCl NaOH Zn dust Sulfamic acid		[38]Revanasiddappa H. D., 2011
7	NIM, pharmaceutical preparations	Resorcinol	Colored azo-dye measured at 480 nm	1.0–40.0	Sulfamic acid Acetone Zink dust HCl $NaNO_3$		[39]Revana-siddappa H. D., 2011
8	LAC NIF, bulk and tablet dosage form	Para Dimethyl Amino Benzaldehyde	Blue color Schiff's base formation measured at 615.7 nm	10.0–70.0	Conc. HCl, heating		[40]Moorthi C., 2011
9	NIM, tablets and injection	Phloro-glucinol	Yellow colored complex measured at 410 nm	0.0–25.0	Acetone HCl Sulfamic acid NaOH $NaNO_3$		[41]Deepakumari, 2013

Table 1 (continued)

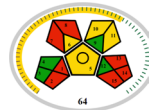
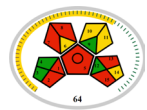
№	Dihydropy-ridine CCBs and sample	Utilized reagents	Conditions	Linearity, µg/mL	Limitation	MOGAPI score	Reference
10	NIF NIM AML, dosage forms	N-bromosucci-nimide, indigo carmine	Bleach the color of the used dye	1.25–13.0	Chloroform Ethylether $HClO_4$		[42]Hamd M., 2013
11	NIF NIM AML, tablets and capsules	Vanillin	Colored red chromo-gen, measured at 479 and 500 nm	5.0–70.0	HCl		[43]Hamd M., 2013
12	NIM NIF, pharmaceutical preparations and spiked human plasma	p-anis-aldehyde	Colored product measured at 460 nm	5.0–60.0	HCl80 ^{0}C		[44]Marzouq M.A., 2015
13	NIF, pharmaceutical formu-lations, serum and urine	3-methyl-2-benzo-thiazolinone hydrazone, brucine	Green colored product measured at 685 nm (I) Violet colored product, measured at 546 nm (II)	1.0–19.0 4.0–18.0 [45] Tula-samma P., 2016	HCl Zinc powder $FeCl_3$ $NaIO_4$		[45]Tula-samma P., 2016
14	AML, tablets	1,2-naphtha-quinone-4-sul-phonic acid sodium salt	Orange colored product measured 459 nm	10.0–20.0	NaOH, heating		[46]Sulyma M., 2018
15	AML, pharmaceutical for-mulation and human urine	Picric acid	Orange colored product measured at 420 nm	100.0–140.0	Dichloro-methane Ace-tonitrile		[47]Kawin Khachornsakkul, 2020
16	NIM AML, bulk, pharmaceu-tical formulation and bio-logical fluids	2,3-dichloro-5,6-dicy-anobenzoquinon	Yellow-orange charge transfer complex measured at 464 nm	1.0–40.0	Borate buffer solution pH = 9 HCl Zinc powder NaOH		[48]Hanan H. Ahmed, 2023
17	NIM, tablets and biological fluids	4-amino-antipyrine	Yellow–brown product measured at 464 nm	1.0–35.0	KIO_3 as oxidization reagent HCl Zinc powder NaOH		[49]Hanan H. Ahmed, 2023

Table 1 (continued)

№	Dihydropy-ridine CCBs and sample	Utilized reagents	Conditions	Linearity, µg/mL	Limitation	MOGAPI score	Reference
18	NIM, pharmaceutical formulation and biological fluids	γ-resorsolic acid	colored azo-dyes measured at 436 nm	1.0–40.0	alkaline medium, HNO_2		[50]Hanan H. Ahmed, 2023

Table 2 Spectrophotometric determination of some CCBs with dyes

№	Dihydropyridines CCB and sample	Utilized reagents	Conditions	Ranges, μg/mL	Remarks	AGREE score	MOGAPI score	Reference
1	AML	BTB	color complex, measured at 417, 416 nm	5.0–40.0	Extraction, $CHCl_3$			[52]Sridhar K., 1997
2	AML	BCG Methyl red	color complexes, measured at 409 nm, 668.2 nm	0.0–80.0	Buffer solution pH = 2.4, 6.8 Extraction Chloroform			[53]Singhvi, 1998
3	NIF	BPB, BTB, BCG and eriochrome black T	Color complexes, measured at 415 and 592 nm	5.0–32.5 4.0–37.5 6.5–33.0 4.5–22.5	Extraction Chloroform HCl Zn dust			[54]Rahman,N., 2004
4	AML	BPB	color complex, measured at 413 nm	4.0–20.0	2.4-acid phthalate buffer solution NaOH Chloroform			[55] Asgar, 2010
5	AML	Amido black	Blue colored ion-pair product, measured at 592 nm	1.0–56.0	Britton buffer (pH = 2.0) Chloroform Extraction			[56]Raghad Alkhalil, 2019
6	AML	Crystal violet	Blue color complex, measured at 592 nm	5.0–92.5	acidic medium potassium bromide: potassium bromate			[57]Al-Mohsen Khdadad, 2022
7	NIM	BTB	Yellow ion-pair product, measured at 414.5 nm	4.0–50.0	Zinc powder Conc. HCl Buffer KCl–HCl pH = 2.0 Chloroform			[58]Nguyen T. D., 2022

spectrophotometric measurements. RAD WAG AS 200/C precise analytical balance (Poland) and ultrasonic bath Elmasonic EASY 60H (Germany) with 100% ultrasonic power at a frequency of 40 kHz were utilized in the developed procedure.

Reagents and standards

Amlodipine besylate (AML), lacidipine (LAC), levamlodipine besylate (LAML), nifedipine (NIF), nimodipine (NIM) and nitrendipine (NIT) (purity ≥ 98% (HPLC)) were supplied from Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA).

Amlodipine besylate 10 mg tablets («Amlodipine» Farmak), Nitrendipine 10 mg tablets («Nitresan» PRO. MED. CS Praha a. s.), Nifedipine 20 mg tablets («Nifedipine» Lekhim), Levamlodipine besylate 5 mg tablets («Semlopin» Kusum Pharm), Nimodipine 30 mg tablets («Nimodipine» PJSC SIC "Borshchahivskiy CPP") were purchased from local drugstore. Lacidipine 2 mg tablets ("Lacipil" GlaxoSmithKline) was purchased from europharm.com.ua.

All used in these studies solvents including methanol, ethanol, chloroform, acetonitrile and ethyl acetate were produced by Honeywell and had a purity of 99.9%. BPB were acquired from Sigma-Aldrich Chemicals Co. (USA, St. Louis). All chemicals utilized in the experiment were analytical grade.

Standard analytical procedure

Preparation of BPB solution for determination of AML and NIF

A 6.0×10^{-4} M of BPB was prepared in a 50.00 mL measuring flask by dissolving 20.76 mg of BPB in 40 mL of methanol and adjusting to the mark with the same solvent.

Preparation of BPB solution for determination of NIT

A 9.0×10^{-4} M of BPB was prepared in a 25.00 mL measuring flask by dissolving 15.6 mg of BPB in 20 mL of methanol and adjusting to the mark with the same solvent.

Preparation of BPB solution for determination of LAML and LAC

A 1.0×10^{-3} M of BPB was prepared in a 25.00 mL measuring flask by dissolving 16.8 mg of BPB in 20 mL of methanol and adjusting to the mark with the same solvent.

Preparation of BPB solution solution for determination of NIM

A 1.0×10^{-3} M of BPB was prepared in a 25.00 mL measuring flask by dissolving 16.8 mg of BPB in 20 mL of acetonitrile and adjusting to the mark with the same solvent.

Preparation of standard solution

Standard stock solutions were prepared by dissolving 25 mg in 15 ml of solvent (methanol for AML, NIF, NIT, LAML/acetonitrile for NIM, LAC), followed by dilution to a final volume in 25 ml vessels, then diluted with suitable solvents to obtain a concentrations 3.40—17.00 μg/mL for AML, 4.16—12.40 μg/mL for NIF, 6.52—19.60 μg/mL for NIT, 1.13—9.08 μg/mL for LAML, 0.84—5.86 μg/mL for NIM, 1.14—9.11 μg/mL for LAC.

Calibration curve construction

Standard stock solutions were transferred to a series of 10 mL volumetric flasks. The volume transferred varied to achieve the desired concentration ranges: (0.1–0.5 mL) for AML, (0.2–0.6 mL) for NIF, (0.2–0.6 mL) for NIT, (0.1–0.8 mL) for LAML, (0.2–1.4 mL) for NIM and (0.05–0.4 mL) for LAC. These concentration ranges allowed for the concentration ranges of 3.40 – 17.00 μg/mL for AML, 4.16 – 12.40 μg/mL for NIF, 6.52 – 19.60 μg/mL for NIT, 1.13 – 9.08 μg/mL for LAML, 0.84 – 5.86 μg/mL for NIM, 1.14 – 9.11 μg/mL for LAC, respectively. The solutions were analyzed by adding BPB 1.0 mL of 6.0×10^{-4} M for AML, NIF, NIT, LAC and 0.3 mL of 1.0×10^{-3} M for LAML, NIM and making up to the mark by solvent. The absorbance of the resulting mixtures were registered at 596 nm against a reference solutions prepared in the same way without adding the analyte.

Application to tablet analysis

Twenty tablets of AML, LAC, LAML, NIF, NIM and NIT were thoroughly crushed and weighed. Sample preparation was carried out as described in Sect. "Preparation of Standard solution" with filtration using a Whatman No. 42 filtering paper.

Results and discussion

Methodology for choosing a reagent for the development of spectrophotometric methods for the determination of dihydropyridines CCBs

The use of sulfophthalein dyes is quite promising in the modern spectrophotometric analysis of drugs. The advantages of consuming these reagents are environmental friendliness, short sample preparation, speed and cost of analysis. Sulfophthalein dyes belong to the acid-basic group and can interact in solution in the form of two tautomeric (hydrogenated) forms, the first- monoanionic, which forms a yellow quinoid structure upon interaction in which the analyte splits off a proton (in the cause of BPB), and the second—dianionic is a blue-colored sulfophthalein decomposition product due to the loss the analyte of one more proton [59]. After reviewing scientific sources, it was established that some of dihydropyridines CCBs have already been studied using sulfophthalein dyes (Table 2) however almost all of them used chloroform extraction and heating, which negatively affects not only sample preparation, increasing the duration of the analysis, but also safety and environmental friendliness. Therefore, our task in the course of preliminary experimental studies was to select the optimal reagent for the development of the spectrophotometric methods for the determination of AML, LAC, LAML, NIF, NIM and NIT in dosage forms without using extraction and heating. To select an effective reagent for the development of the spectrophotometric procedure for the determination of dihydropyridines CCBs were tested a number of sulfophthalein dyes: BPB, BCG, BTB, bromocresol purple (BCP), thymol blue (TB), cresol red (CR) and phenol red (PR) (Table 3). We were not interested in the UV part of the spectrum, therefore, in some figures are observed the truncated absorption maxima of the reaction products.

Methodology for choosing a reagent for the development of the spectrophotometric methods for the determination of AML in dosage forms.

In order to select the optimal reagent for the development of the spectrophotometric method for the determination of AML in dosage forms were tested BCG, BTB, BPB, BCP, PR, CR, TB. Fig. S. 3.1.1 (Appendix A) shows the absorbance spectra obtained during the approbation of BCG as a potential reagent for the spectrophotometric procedure of the determination of AML. As can be seen from Fig. S. 3.1.1 (Appendix A), BCG and AML interact and form a product with an absorbance maximum at a wavelength of 350 nm. Methanol was chosen as the optimal solvent. For further method development the necessary was pH correction, which complicate sample preparation and negatively affect the "greenness" of the method. Fig. S. 3.1.2 (Appendix A) illustrated the absorbance spectra obtained when BTB was used as a potential reagent for the spectrophotometric method of the determination of AML. As can be seen from Fig. S. 3.1.2 (Appendix A) a shift of the formed complex with a maximum at a wavelength of 350 nm was observed, while the complex and BTB against acetonitrile was registered at 405 nm. The obtained results required a change of pH, as with the previous BCG reagent. The next dye that we tested for the development of the spectrophotometric method for the determination of AML was BPB. As a result of the interaction of AML with BPB was formed

Table 3 Methodology for choosing a reagent for the development of the spectrophotometric methods for the determination of dihydropyridines CCBs

Analyte	Reagent	Optimal conditions (wavelenght, solvent, molar concentration)	Possibility of application	Remark
AML	BCG	415 nm, MetOH, $5.0*10^{-4}$ M	No	pH adjustment
	BTB	405 nm, MetOH, $2.49*10^{-4}$ M	No	pH adjustment
	BPB	598 nm, MetOH, $6.0*10^{-5}$ M	Yes	Methanol medium, absorption maximum at a wavelength 598 nm
	BCP	415 nm, EtAc, $2.26*10^{-4}$ M	No	pH adjustment
	CR	398 nm, AcN, $2.5*10^{-4}$ M	No	pH adjustment
	PR	350 nm, AcN, $1.0*10^{-4}$ M	No	No interaction
	TB	405 nm, AcN, $1.0*10^{-4}$ M	No	pH adjustment
NIF	BCG	405 nm, MetOH, $5.0*10^{-4}$ M	No	pH adjustment
	BTB	398 nm, MetOH, $2.76*10^{-4}$ M	No	pH adjustment
	BPB	598 nm, MetOH, $5.0*10^{-4}$ M	Yes	Methanol medium, absorption maximum at a wavelength 598 nm
	BCP	415 nm, EtAc, $2.26*10^{-4}$ M	No	pH adjustment
	CR	380 nm, AcN, $2.5*10^{-4}$ M	No	pH adjustment
	PR	350 nm, AcN, $2.26*10^{-4}$ M	No	No interaction
NIT	BCG	415 nm, MetOH, $5.0*10^{-4}$ M	No	pH adjustment
	BTB	350 nm, MetOH, $2.77*10^{-5}$ M	No	pH adjustment
	BPB	598 nm, metOH, 5,0*10–4 M	Yes	Methanol medium, absorption maximum at a wavelength 598 nm
	BCP	415 nm, EtAc, $2.26*10^{-4}$ M	No	No interaction
	CR	398 nm, AcN, $2.5*10^{-4}$ M	No	pH adjustment
	TB	350 nm, AcN, $1.0*10^{-4}$ M	No	No interaction
NIM	BTB	423 nm, MetOH, $5.0*10^{-5}$ M	No	pH adjustment
	BPB	598 nm, AcN	Yes	Acetonitrile medium, absorption maximum at a wavelength 598 nm
	BCP	420 nm, MetOH, $5.0*10^{-5}$ M	No	pH adjustment
	CR	423 nm, EtOH, $1.0*10^{-4}$ M	No	pH adjustment
	TB	390 nm, AcN, $1.0*10^{-4}$ M	No	pH adjustment
LAC	BCG	MetOH, $1*10^{-3}$ M	No	No interaction
	BTB	MetOH, $1.0*10^{-3}$ M	No	No interaction
	BPB	AcN, $1.0*10^{-3}$ M	Yes	Acetonitrile medium, absorption maximum at a wavelength 598 nm
	BCP	MetOH, $1.0*10^{-3}$ M	No	No interaction
	MR	AcN, $1.0*10^{-3}$ M	No	No interaction
	CR	MetOH, $1.0*10^{-3}$ M	No	No interaction
	PR	MetOH, $1.0*10^{-3}$ M	No	No interaction
	TB	MetOH, $1.0*10^{-3}$ M	No	No interaction
LAML	BCG	MetOH, $1.0*10^{-3}$ M	No	No interaction
	BTB	MetOH, $1.0*10^{-3}$ M	No	No interaction
	BPB	MetOH, $1.0*10^{-3}$ M	Yes	Methanol medium, absorption maximum at a wavelength 598 nm
	BCP	MetOH, $1.0*10^{-3}$ M	No	No interaction
	MR	AcN, $1.0*10^{-3}$ M	No	No interaction
	CR	MetOH, $1.0*10^{-3}$ M	No	No interaction
	PR	MetOH, $1.0*10^{-3}$ M	No	No interaction
	TB	MetOH, $1.0*10^{-3}$ M	No	No interaction

a reaction product with an absorbance maximum at a wavelength of 596 nm. Adding AML to the BPB solution decreases the absorbance band of the monoionized dye form while increases the absorbance band of the doubly ionized form. Absorbance spectra of the reaction product of AML with BPB are presented in Fig. S. 3.1.3 (Appendix A). Considering the results received in Fig. S. 3.1.3, we were interested in BPB as a reagent for the further method development. Fig. S. 3.1.4 (Appendix A) demonstrated the absorbance spectra obtained during the approbation of BCP as a potential reagent for the development of a spectrophotometric procedure

for the determination of AML. Ethyl acetate was chosen as the optimal solvent. As can be seen from Fig. S. 3.1.4 (Appendix A), the interaction of BCP and AML produced a product with an absorbance maximum at a wavelength of 370 nm, however, similarly to the previous dyes BCG and BTB need to adjust the pH, which was inappropriate for routine drug analysis. Fig. S. 3.1.5 (Appendix A) illustrates the absorbance spectra of the reaction product of AML with the next tested sulfophthalein dye—PR. The achived results from Fig. S. 3.1.5 (Appendix A) indicate that AML does not interact with PR however the absorbance of the dye was greater than that of the complex against acetonitrile. Fig. S. 3.1.6 (Appendix A) shows the absorbance spectra obtained when testing CR as a potential reagent for the determination of AML. Similarly, as with BCG, BTB, BCP, further development of the methodology required the pH adjustment, which complicates sample preparation. The last tested sulfophthalein dye as a potential reagent for the development of the spectrophotometric method for the determination of AML was TB. The absorbance spectra of the reaction product in acetonitrile are shown in Fig. S. 3.1.7 (Appendix A). It turned out to be interesting that TB interacted better with AML in a methanol–acetonitrile solution. As can be seen from Fig. S. 3.1.8 (Appendix A), the maximum absorbance of the product of the interaction of AML with TB in the methanol–acetonitrile medium was observed at a wavelength of 370 nm, while in acetonitrile (Fig. S. 3.1.7 (Appendix A)) at 350 nm. The solvatochromic effect of organic solvents leads to a shift in the position of these forms. However, in subsequent studies, problems arose with the linearity of the analytical method, which may be related to the need to adjust the pH. Therefore, from all the above-mentioned sulfophthalein dyes, BPB was chosen for the method further development of AML in dosage forms.

Methodology for choosing a reagent for the development of the spectrophotometric methods for the determination of NIF in dosage forms

In order to select the optimal reagent for the development of the spectrophotometric method for the determination of NIF in dosage forms were tested BCG, BTB, BPB, BCP, PR, CR. Fig. S. 3.2.1 (Appendix B) shows the absorbance spectra obtained during the approbation of BCG as a potential reagent for the spectrophotometric method of determining NIF. As shown in Fig. S. 3.2.1 (Appendix B), BCG interacts with NIF and forms a product with an absorbance maximum at a wavelength of 350 nm. Methanol was chosen as the optimal solvent. However, it was necessary for the further method development to adjust the pH, which leads to the complication of sample preparation and negatively affect the "greenness" of the method. Fig. S. 3.2.2 (Appendix B) demonstrated the absorbance spectra obtained when BTB were used as a potential reagent for the spectrophotometric procedure of the determination NIF. As can be seen from Fig. S. 3.2.2 (Appendix B), the formation of the reaction product of NIF and BTB was not observed, so it could be concluded that NIF did not interact with BTB. Absorbance spectra of the reaction product of NIF and potential reagent BPB presented in Fig. S. 3.2.3 (Appendix B). NIF interacts with BPB with formation of a complex with an absorbance maximum at a wavelength of 596 nm. Adding NIF to the BPB solution decreases the absorbance band of the monoionized dye form while increases the absorbance band of the doubly ionized form. Considering the results obtained in Fig. S. 3.2.3 (Appendix B), we were interested in BPB as a reagent for the further method development. Fig. S. 3.2.4 (Appendix B) shows the absorbance spectra obtained when BCP was tested as a potential reagent for the method development. Ethyl acetate was chosen as the optimal solvent. As can be seen from Fig. S. 3.2.4 (Appendix B), BCP did not react with NIF, similarly to BTB. Fig. S. 3.2.5 (Appendix B) illustrated the absorbance spectra obtained during the approbation of PR as a potential reagent for the development of the spectrophotometric procedure for the determination of NIF. As can be seen from Fig. S. 3.2.5 (Appendix B), NIF did not interact with PR, the same conclusion as for previously tested reagents BTB, BCP. Fig. S. 3.2.6 (Appendix B) shows the absorbance spectra obtained when testing CR as a potential reagent for the determination of NIF. As can be seen from Fig. S. 3.2.6 (Appendix B), the further development of the method involves adjusting the pH, which increases the duration of the analysis and affect the «greeness» of the method. From the above mentioned, it can be concluded that among all tested sulfophthalein dyes, BPB was chosen as a potential reagent for the development of the spectrophotometric method for the determination of NIF in dosage forms.

Methodology for choosing a reagent for the development of spectrophotometric methods for the determination of NIT in dosage forms

In order to select the optimal reagent for the development of the spectrophotometric method for the determination of NIT in dosage forms were tested BCG, BTB, BPB, BCP, CR, TB. Fig. S. 3.3.1 (Appendix C) shows the absorbance spectra obtained during the approbation of BCG as a potential reagent for the spectrophotometric method of determining NIT. As shown in Fig. S. 3.3.1 (Appendix C), BCG interacts with NIT and forms a product with an absorbance maximum at a wavelength of 350 nm. Methanol was chosen as the optimal solvent.

However, it was necessary for the further development of the method to adjust the pH, which leads to the complication of sample preparation and negatively affect the "greenness" of the analytical method. Fig. S. 3.3.2 (Appendix C) illustrated the absorbance spectra obtained during the approbation of BTB as a potential reagent for the spectrophotometric method of determination NIT. As can be seen from Fig. S. 3.3.2 (Appendix C), the formation of the product of the reaction of NIT and BTB was observed. However, for further method development required the adjustement the pH, similarly to BCG. Absorbance spectra of the reaction product of NIT when testing a potential reagent BPB are presented in Fig. S. 3.3.3 (Appendix C). NIT interacts with BPB with formation of the reaction product with an absorbance maximum at a wavelength of 596 nm. Adding NIT to the BPB solution decreases the absorbance band of the monoionized dye form while increases the absorbance band of the doubly ionized form. Considering the results obtained in Fig. S. 3.3.3 (Appendix C), we were interested in BPB as a reagent for the further method development. Fig. S. 3.3.4 (Appendix C) shows the absorbance spectra obtained during the approbation of BCP as a potential reagent for the development of a method for the spectrophotometric determination of NIT. Ethyl acetate was chosen as the optimal solvent. As can be seen from Fig. S. 3.3.4 (Appendix C), BCP did not react with NIT. Fig. S. 3.3.5 (Appendix C) illustrates absorbance spectra obtained using CR as a potential reagent for the determination of NIT. As can be seen from Fig. S. 3.3.5 (Appendix C), the further method development involves adjusting the pH, which leads to an increase in the duration of the analysis and will affect environmental friendliness methods, the same conclusion as for previously tested BCG and BTB dyes. The last tested sulfophthalein dye as a potential reagent for the development of the spectrophotometric method for the determination of NIT was TB. The absorbance spectra of the reaction product in acetonitrile are shown in Fig. S. 3.3.6 (Appendix C). NIT did not interact with TB, similarly to BCP. So, from all tested sulfophthalein dyes, BPB was chosen as a potential reagent for the development of the spectrophotometric method for the determination of NIT in dosage forms.

Methodology for choosing a reagent for the development of the spectrophotometric methods for the determination of NIM in dosage forms

In order to choose the optimal reagent for the development of the spectrophotometric method for the determination of NIM in dosage forms were tested BTB, BPB, BCP, CR, TB. Fig. S. 3.4.1 (Appendix D) presented absorpbance spectra obtained during the testing of BTB as a potential reagent for the development of the spectrophotometric method for the determination of NIM. As can be seen from Fig. S. 3.4.1 (Appendix D), NIM interacts with BTB however this technique requires pH adjustment, which negatively affects the aspects described above. Fig. S. 3.4.2 (Appendix D) shows the absorbance spectra obtained during the testing of BCP as a potential reagent for the development of a method for the determination of NIM. Methanol was chosen as the optimal solvent. As can be seen from Fig. S. 3.4.2 (Appendix D), a high value of the absorbance was observed in the spectra. Since BCP did not interact with NIM, we did not dilute the solutions to obtain a satisfactory value of the absorbance. Fig. S. 3.4.3 (Appendix D) shows the absorbance spectra obtained during the approbation of CR as a potential reagent for the method development. As can be seen from Fig. S. 3.4.3 (Appendix D), NIM interacts with CR however requires pH adjustment, like previously tested BTB. The absorpbance spectra of the reaction product of NIM with TB in acetonitrile are shown in Fig. S. 3.4.4 (Appendix D). NIM interacts with TB however further development of the method requires changing the pH of the medium, the same conclusion as for previously tested dyes BTB and CR. Therefore, among the above-mentioned sulfophthalein dyes, BPB Fig. S. 3.4.5 (Appendix D) was a potential reagent for the development of the spectrophotometric method for the determination of NIM in dosage forms.

Methodology for choosing a reagent for the development of the spectrophotometric methods for the determination of LAC in dosage forms

Taking into consideration the bank of negative results obtained during the investigation of the previous four analytes with sulfophthalein dyes, we decided that it is impractical to conduct further studies with the consumption of a large number of solvents, test substances and dyes. While next developing the further methodology for the selection of reagents, we changed the methodology and approach to the selection of reagents and began to use the « plate method», which significantly saved our time. A visual method was proposed for assessing the interaction of the analyte with the dye using a plate. Fig. S. 3.5.1 (Appendix E) shows a plate with experimental data on the interaction of LAC and sulfophthalein dyes, as potential reagents (TB, methyl red (MR), CR, PR, BCP, BCG, BPB, BTB). A blank solution was added to columns 1, 3, and 5, which consisted of 1 drop of dye solution + 1 drop of methanol + 13 drops of solvent (1-MetOH, 3-EtOH, 5-ACN). In columns 2, 4 and 6 were added the test solution, which consisted of 1 drop of dye solution + 1 drop of LAC solution + 13 drops of solvent (1-MetOH, 3-EtOH, 5-ACN). As can be seen from Fig. S. 3.5.1 (Appendix E), the color change in compartment G

indicates the formation of a complex of LAC with BPB. All other tested dyes did not interact with LAC under normal conditions, since the color of the blank solution did not differ from the complex or was more intense.

Methodology for choosing a reagent for the development of the spectrophotometric methods for the determination of LAML in dosage forms

A visual method was proposed of choosing a potential reagent for the development of a spectrophotometric method for the determination of LAML in dosage forms using a plate. Fig. S. 3.6.1 (Appendix F) shows a plate with experimental data on the interaction of LAML and sulfophthalein dyes, as potential reagents (TB, MR, CR, PR, BCP, BCG, BPB, BTB). A blank solution was added to columns 1, 3, and 5, which consisted of 1 drop of dye solution + 1 drop of methanol + 13 drops of solvent (1-MetOH, 3-EtOH, 5-ACN). In columns 2, 4 and 6 were added the test solution, which consisted of 1 drop of dye solution + 1 drop of LAML solution + 13 drops of solvent (1-MetOH, 3-EtOH, 5-ACN). As can be seen from Fig. S. 3.6.1 (Appendix F), the color change in compartment G indicates the formation of a complex of LAML with BPB. All other tested dyes did not interact with LAML under normal conditions, since the color of the blank solution did not differ from the complex or was more intense.

Selection the optimal conditions for the development of the spectrophotometric methods for the determination of dihydropyridines CCBs

As it was mentioned in Sect. 3.1, the spectrophotometric determination based on ion pair formation are among the most important optical instrumental quantitative methods and warrant special attention due to their prominent application in drug quantification. Sulfophthaleins are one of the most interesting dye families in the development of such techniques and they have drawn scientific interest because of their molecular structure, which enables the formation of ion-pair complexes with a variety of drugs and their anionic nature even at acidic pH due to the presence of a sulfonic group in its dissociated form [59]. It was shown that some CCBs drugs can be spectrophotometrically determined using BPB as for ion-pair complexes formation [54, 55]. Scientific publications [54, 55] describe the methods for the determination of AML and NIF using BPB however the use of chloroform as a toxic solvent and an analytical wavelength of 413–415 nm (monoanionic form) were required, which indicated the outdated approaches in the development of methods due to the time of development of methods (2004, 2010). BPB is a dye that has been used as an industrial dye, a laboratory indicator and a biological stain. BPB

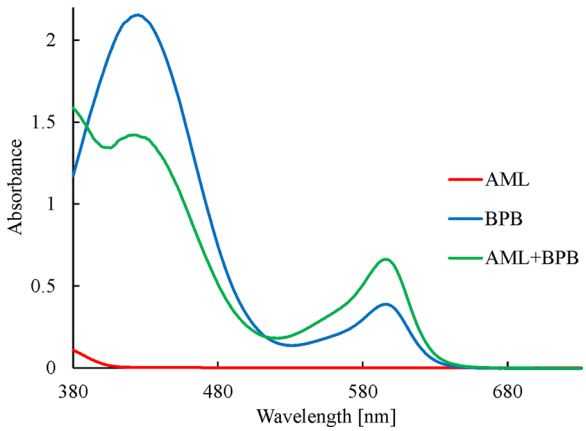


Fig. 2 Absorbance spectra of AML, BPB and AML—BPB complex *vs.* appropriate solvent (c(BPB) = 6.00×10^{-5} M, c(AML) = 1.80×10^{-5} M)

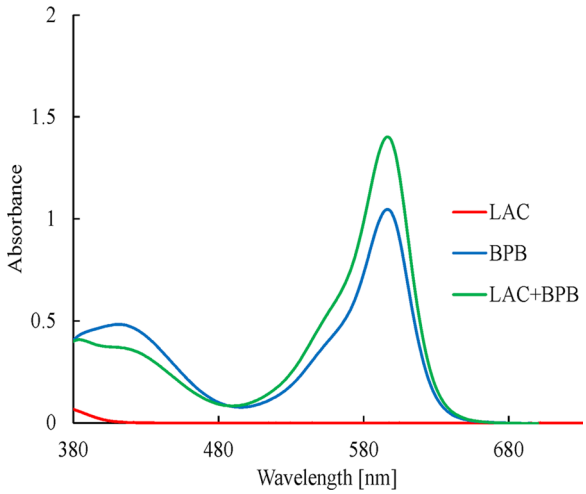


Fig. 3 Absorbance spectra of LAC, BPB and LAC—BPB complex *vs.* appropriate solvent (c(BPB) = 3.00×10^{-5} M, c(LAC) = 1.00×10^{-5} M)

is not a hazardous substance and can be used as reagent for «green» analysis. It has a role as a two-colour indicator, an acid–base indicator, changing from yellow below pH 3 to purple at pH 4.6 [60]. The absorbance spectra of the BPB complex with several CCBs, such as AML, LAC, LAML, NIF, NIM and NIT, are displayed in Figs. 2, 3, 4, 5, 6, 7. The absorbance maxima of these drugs were located at 596 nm.

This approach was important as it avoids the need for an extraction step, which was the primary motivation behind developing this analytical procedure. Some experimental settings for the reaction must be tuned in order to establish the optimal spectrophotometric conditions for the analysis of CCBs drugs using reaction with BPB. Correct selection of organic solvents

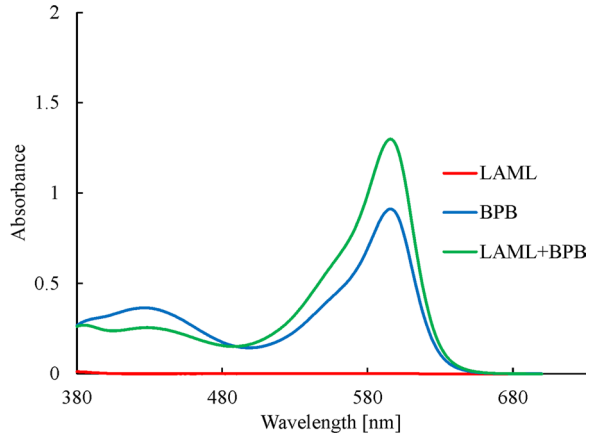


Fig. 4 Absorbance spectra of LAML, BPB and LAML—BPB complex *vs.* appropriate solvent (c(BPB) = 3.00 × 10^{-5} M, c(LAML) = 1.40 × 10^{-5} M)

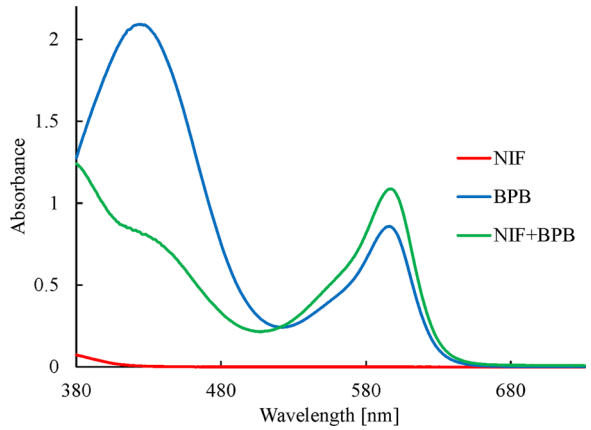


Fig. 5 Absorbance spectra of NIF, BPB and NIF—BPB complex *vs.* appropriate solvent (c(BPB) = 6.00 × 10^{-5} M, c(NIF) = 2.40 × 10.^{5} M)

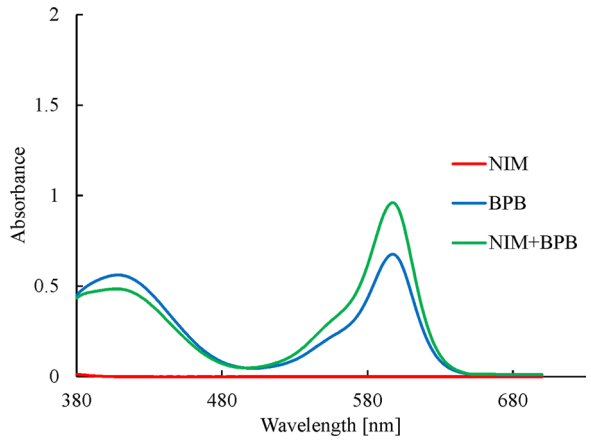


Fig. 6 Absorbance spectra of NIM, BPB and NIM—BPB complex *vs.* appropriate solvent (c(BPB) = 3.00 × 10^{-5} M, c(NIM) = 1.00 × 10^{-5} M)

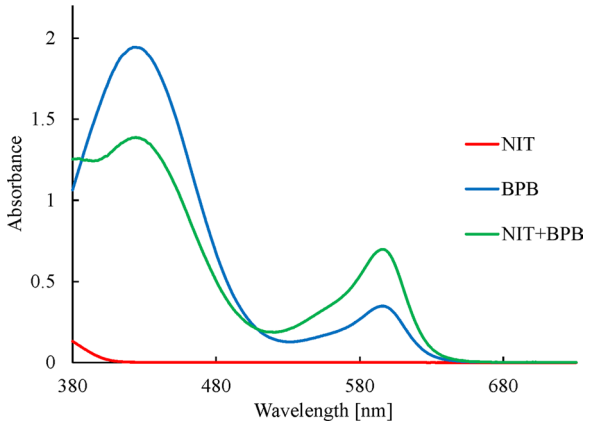


Fig. 7 Absorbance spectra of NIT, BPB and NIT—BPB complex *vs.* appropriate solvent (c(BPB) = 9.02 × 10^{-5} M, c(NIT) = 3.64 × 10^{-5} M)

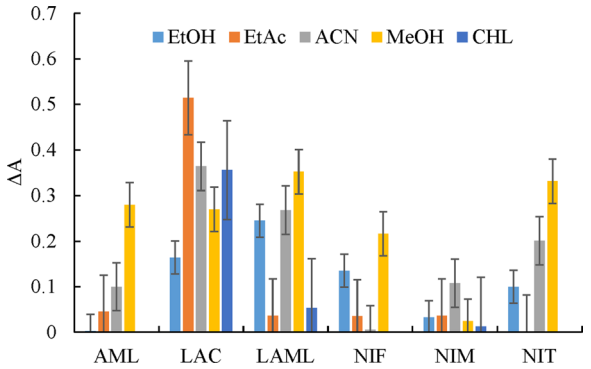


Fig. 8 Effects of solvents on CCBs drugs—BPB complex formation (AML (c(BPB) = 6.00 × 10^{-5} M, c(AML) = 1.80 × 10^{-5} M), LAC (c(BPB) = 5.00 × 10^{-5} M, c(LAC) = 2.50 × 10^{-5} M), LAML (c(BPB) = 2.00 × 10^{-5} M, c(LAML) = 2.00 × 10^{-6} M), NIF (c(BPB) = 6.00 × 10^{-5} M, c(NIF) = 2.40 × 10^{-5} M), NIM (c(BPB) = 3.00 × 10^{-5} M, c(NIM) = 2.00 × 10^{-6} M), NIT (c(BPB) = 3.64 × 10^{-5} M, c(NIT) = 9.02 × 10^{-5} M)

was crucial as it facilitates and guarantees the interaction between the analyte and BPB, resulting in the production of the appropriate colored product. For the purpose of comparing solvents, acetonitrile, chloroform, methanol,ethanol and ethyl acetate were used. The solvent selection results are shown in Fig. 8. For AML, LAML, NIF and NIT methanol was considered the optimal solvent, whereas for NIM it was acetonitrile. LAC-BPB complex achieves the highest absorbance in ethylacetate however the solution was stable for less than 5 min, so we recommended acetonitrile as a solvent for LAC determination too. The influence of dye concentration on the absorption increasing of its complex with LAC at the selected analytical wavelength was studied. It was demonstrated that the optimal BPB

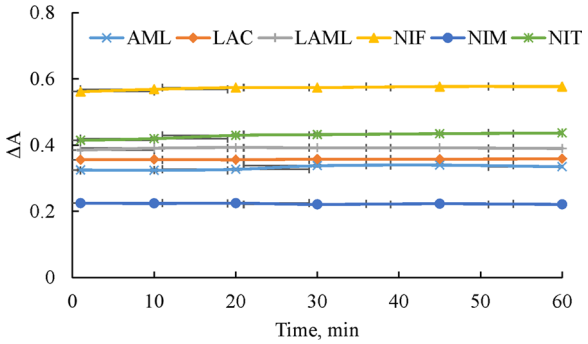


Fig. 9 Stability of some CCBs drugs complexes with BPB in time (AML ($c(BPB) = 6.00 \times 10^{-5}$ M, $c(AML) = 2.40 \times 10^{-5}$ M), LAC ($c(BPB) = 3.00 \times 10^{-5}$ M, $c(LAC) = 1.00 \times 10^{-5}$ M), LAML ($c(BPB) = 3.00 \times 10^{-5}$ M, $c(LAML) = 1.40 \times 10^{-5}$ M), NIF ($c(BPB) = 6.00 \times 10^{-5}$ M, $c(NIF) = 6.00 \times 10^{-5}$ M), NIM ($c(BPB) = 3.00 \times 10^{-5}$ M, $c(NIM) = 1.00 \times 10^{-6}$ M), NIT ($c(BPB) = 9.02 \times 10^{-5}$ M, $c(NIT) = 4.55 \times 10^{-5}$ M)

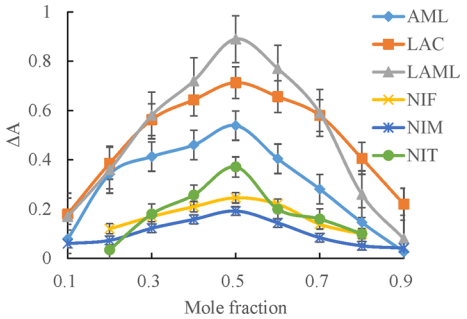


Fig. 10 Study of stoichiometric coefficients by the reaction of selected CCBs with BPB at λmax = 596 nm, using Job's method of continuous variations (AML ($c(BPB) = 6.00 \times 10^{-4}$ M, $c(AML) = 6.00 \times 10^{-4}$ M), LAC ($c(BPB) = 5.00 \times 10^{-4}$ M, $c(LAC) = 5.00 \times 10^{-4}$ M, C% MeOH = 8%), LAML ($c(BPB) = 1.00 \times 10^{-4}$ M, $c(LAML) = 1.00 \times 10^{-4}$ M), NIF ($c(BPB) = 6.00 \times 10^{-4}$ M, $c(NIF) = 6.00 \times 10^{-4}$ M), NIM ($c(BPB) = 5.00 \times 10^{-4}$ M, $c(NIM) = 5.00 \times 10^{-4}$ M), NIT ($c(BPB) = 9.02 \times 10^{-4}$ M, $c(NIT) = 9.10 \times 10^{-4}$ M)

concentration was 3.00×10^{-5} M (Fig. S 3.7.1 (Appendix G)). When methanol was present in the reaction mixture during LAC determination, absorption increases. The ideal concentration of methanol was found to be 8% (Fig. S 3.7.2 (Appendix G)).

The stability of the analyzed CCBs and BPB ion-pair complexes was examined. The mixture was kept at a constant temperature of 25 ± 2 °C for at least five minutes and remained stable for at least an hour, even though the ion pairs formed immediately (Fig. 9).

The isomolar series and molar ratios approach were used to calculate the stoichiometric coefficients of the reaction between studied CCBs and BPB. The isomolar series technique (Job's method) is one of the most well-liked and often applied approaches for determining the ratio of components. The primary idea behind Job's approach is to combine the components under study in various ratios while maintaining a constant volume ratio. The drug-dye complex's stoichiometry was determined to be 1:1 (Fig. 10).

The ratio of reacting components as 1 to 1 was also confirmed by the method of molar ratios. Molar ratios are a useful tool for analyzing the relationship between the concentration of one solution component's absorption and the constant concentration of another (first curve) and the amount of the second component's absorption that the first component's constant concentration (second curve) depends on. A saturation curve intersecting at the coefficient of the variable-concentration component is equivalent to that, as shown in Fig. 11.

The optimal parameters for CCBs spectrophotometric analysis via complex formation using BPB were as follows: detection wavelength—596 nm, reaction time—5 min, ratio of reacting components—1:1, operating temperature—25 ± 2 °C. The optimal solvent and BPB concentration are presented in **Table S1** (Appendix G).

Validation of the spectrophotometric methods for the determination of dihydropyridines CCBs

The proposed spectrophotometric methods for determination of studied CCBs in tablets have been validated for the following parameters: robustness, accuracy and precision, linearity, and range of application, in accordance with the requirements of the guidelines of the International Conference on Harmonization (ICH) [61]. Robustness (reaction time, reagent volume change, and absorbance stability) was assessed during the method's development. Previous development studies have shown that changes performed throughout the robustness research within ± 10% do not significantly change the absorbance value (Table 4). All calculated results fall between 98.0% and 102.0% and met the acceptance criteria. Regression analysis was used to assess the linearity of the proposed spectrophotometric methods for the determination of CCBs by reaction with BPB at λ_{max} 596 nm. Linearity results for the assessed spectrophotometric procedures are shown in Table 5. It was shown that determination of AML had the broadest range. The correlation coefficients were higher than 0.998 for all analytes, indicating that all of the analytical procedures linearity was acceptable. Three drug concentration levels were examined in order to evaluate the accuracy of the proposed methods. The obtained results showed a significant agreement between the measured and real ones, as shown in Table 6, proving the correctness of the established procedures. The average recovery percentage varies between 99.0% and 101.0%. A recovery

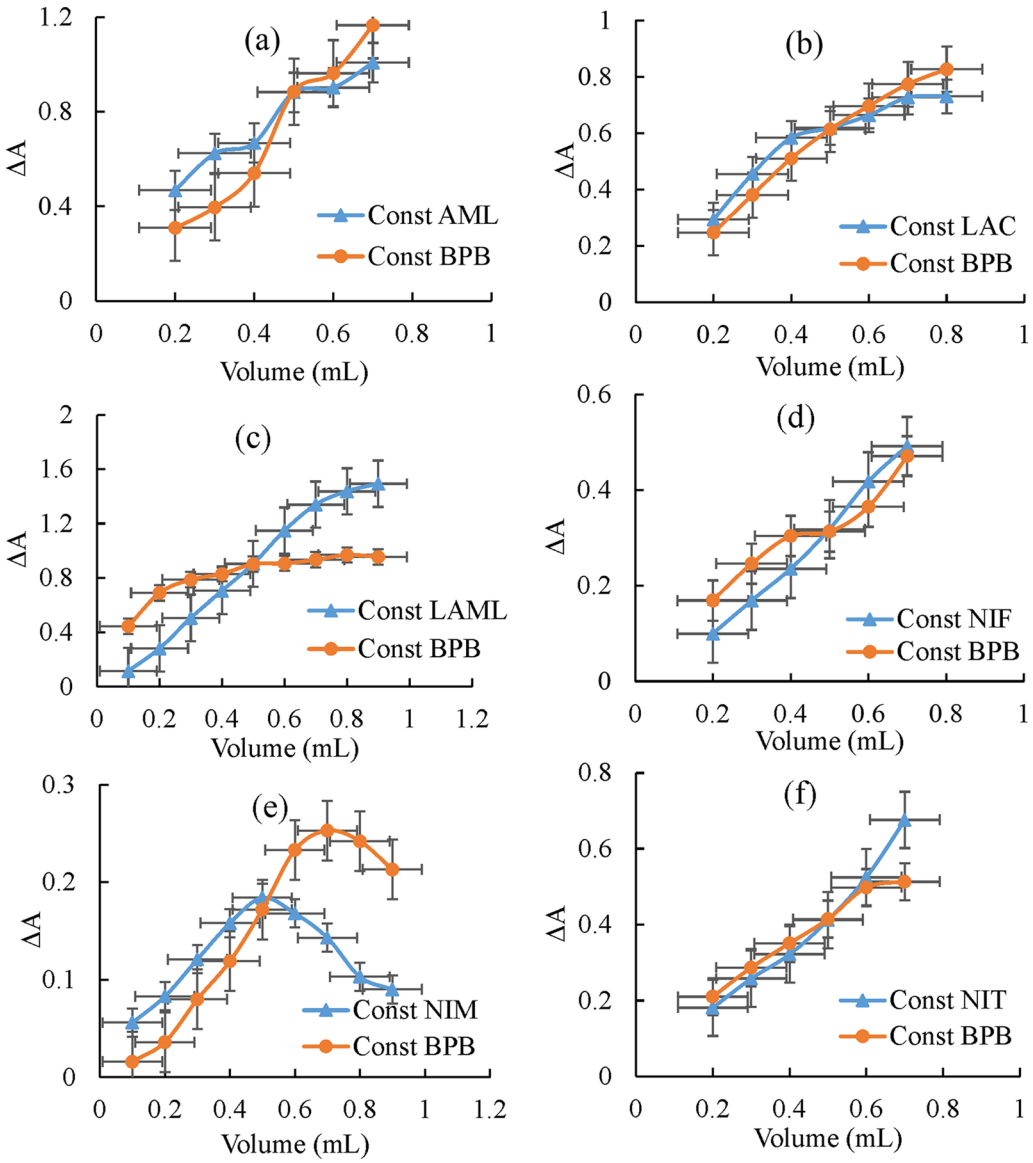


Fig. 11 Study of stoichiometric coefficients by the reaction of selected CCBs with BP B at λmax = 596 nm, using molar ratio method: (**a**)—AML (c(BPB) = 6.00×10^{-4} M, c(AML) = 6.00×10^{-4} M); (**b**)—LAC (c(BPB) = 5.00×10^{-4} M, c(LAC) = 5.00×10^{-4} M, C% MeOH = 8%); (**c**)—LAML (c(BPB) = 1.00×10^{-4} M, c(LAML) = 1.00×10^{-4} M); (**d**)—NIF (c(BPB) = 6.00×10^{-4} M, c(NIF) = 6.00×10^{-4} M); (**e**)—NIM (c(BPB) = 5.00×10^{-4} M, c(NIM) = 5.00×10^{-4} M); (**f**)—NIT (c(BPB) = 9.02×10^{-4} M, c(NIT) = 9.10×10^{-4} M)

value between 98.0% and 102.0% was assigned to each concentration level. For each recovery value, the RSD was less than 1.28%. The degree to which the experimental outcomes agreed with one another was assessed using intra- and inter-day precision evaluation. Inter-day precision was attained by tracking the same concentrations for three days in a row, whereas intra-day precision was attained by repeating the measurement of three analyte concentration levels at three different times of the day. The obtained results are displayed in Table 7.

Application to tablet analysis

The suggested methods were effectively employed to determine the studied CCBs in their tablet dosage forms. Table 8 demonstrates favorable outcomes indicating high accuracy. The developed methods are colorimetric and therefore does not require a UV instrument to quantify these drugs. The proposed methods can be used to control the quality of dosage forms, study the stability and impact of changes on dosage forms while implementation of new manufacturers of excipients, when changing manufacturing technology, changing the composition

Table 4 Robustness of the proposed methods

Method parameters	Recovery* ± SD					
	AML	LAC	LAML	NIF	NIM	NIT
Stability of solutions, min						
15	100.21 ± 1.5	100.92 ± 1.11	100.12 ± 1.23	101.01 ± 0.69	99.87 ± 0.98	100.12 ± 0.93
30	99.97 ± 1.56	99.78 ± 0.77	100.69 ± 1.09	98.86 ± 0.36	100.09 ± 1.42	100.42 ± 0.98
60	99.69 ± 0.88	101.21 ± 0.39	99.5 ± 0.4	100.56 ± 0.09	99.53 ± 1.13	100.14 ± 1.3
Volume of reagent, mL						
0.9	100 ± 1.54	100.81 ± 0.66	99.62 ± 1.43	100.93 ± 0.83	100.12 ± 0.93	99.97 ± 0.8
1.0	98.86 ± 0.46	99.9 ± 1.29	100.68 ± 0.55	99.43 ± 0.66	100.42 ± 0.98	100.16 ± 1.03
1.1	98.91 ± 0.73	100.12 ± 0.98	99.5 ± 0.91	100.25 ± 1.55	100.14 ± 1.3	100.3 ± 1.22
Time of reaction, min						
5	100.12 ± 1.45	100.76 ± 1.26	100.23 ± 1.77	98.9 ± 0.73	99.85 ± 1.35	100.51 ± 1.2
10	99.8 ± 1.34	100.44 ± 0.98	99.53 ± 1.36	99.81 ± 0.59	100.83 ± 1.25	99.11 ± 0.76
15	99.35 ± 0.76	99.64 ± 0.91	100.54 ± 1.48	100.55 ± 0.48	99.7 ± 1.02	99.27 ± 1.3

*Average of the three results

Table 5 Linearity results for the evaluated spectrophotometric procedures

Parameter	AML	LAC	LAML	NIF	NIM	NIT
	Value					
Linearity, µg/mL	**3.40 – 17.00**	**1.14 – 9.11**	**1.135 – 9.08**	**4.16 – 12.40**	**0.84 – 5.86**	**6.52 – 19.60**
Correlation coefficient (R^2)	0.9996	0.9992	0.9992	0.9996	0.9993	0.9980
Intercept ± SD*	$0.0913 \pm 2.29 \times 10^{-3}$	$-0.0077 \pm 7.37 \times 10^{-3}$	$-0.0358 \pm 5.11 \times 10^{-3}$	$0.0056 \pm 2.60 \times 10^{-3}$	$0.0274 \pm 2.66 \times 10^{-3}$	$0.0711 \pm 7.92 \times 10^{-3}$
Slope ± SD	$0.0173 \pm 2.03 \times 10^{-4}$	$0.0791 \pm 1.32 \times 10^{-3}$	$0.0754 \pm 8.91 \times 10^{-4}$	$0.0255 \pm 2.95 \times 10^{-4}$	$0.0255 \pm 7.12 \times 10^{-4}$	$0.0215 \pm 5.72 \times 10^{-4}$

* SD: Standard Deviation

Table 6 Accuracy assessment of the proposed spectrophotometric determinations of CCBs using BPB

Conc level	AML	LAC	LAML	NIF	NIM	NIT
	% Recovery*					
1	99.76	100.57	99.44	100.47	99.64	98.3
2	98.76	99.94	100.68	100.43	99.53	98.92
3	100.08	99.94	100.26	100.02	99.31	100.67
*Mean Recovery	99.53	100.15	100.13	100.31	99.5	99.29
SD	1.07	1.23	1.17	1.21	1.25	1.28

* Mean: of three parallel determinations

of drugs and other processes of the life cycle of dosage forms of studied CCBs**.** However, the proposed methods have limitations. It is not possible to use developed methods for CCBs determination in the presence of other APIs which may affect the analytical reaction.

Greenness assessment

While the developing analytical methods for the quantification, an important aspect is the implementation of the principles of «green» chemistry, which minimize the negative impact of chemicals on the environment and human health [62–66]. «Green» chemistry is a philosophy of modern chemical research, which is aimed at the use of renewable raw materials, the elimination of extraction, the use of small quantities of reagents and a tested

Table 7 Intra- and inter-day precision assessment

Analyte	Concentration level	% Mean Recovery* ± SD	
		Intra-day	Inter-day
AML	1	100.18 ± 1.43	100.62 ± 0.85
	2	99.24 ± 0.69	100.03 ± 1.64
	3	100.55 ± 1.48	99.75 ± 1.52
LAC	1	100.93 ± 0.79	99.41 ± 1.42
	2	100.44 ± 0.98	100.86 ± 0.63
	3	100.12 ± 1.54	100.53 ± 1.27
LAML	1	99.98 ± 0.71	99.5 ± 0.37
	2	99.84 ± 0.66	99.31 ± 1.02
	3	99.47 ± 1.15	100.23 ± 1.51
NIF	1	98.88 ± 0.54	101.08 ± 0.77
	2	99.86 ± 0.89	99.1 ± 0.63
	3	100.2 ± 1.81	99.77 ± 1.36
NIM	1	100.37 ± 1.13	100.56 ± 0.99
	2	99.81 ± 1.27	100.29 ± 1.41
	3	100.75 ± 1.14	99.94 ± 1.16
NIT	1	99.62 ± 1.79	99.9 ± 1.52
	2	100.51 ± 1.14	99.98 ± 1.15
	3	98.96 ± 0.24	100.54 ± 1.5

* Mean of three measurements

sample, the use of catalysts to increase efficiency, reduce waste.

Scientists use a variety of tools and approaches to calculate the «greenness», one of which is the Analytical GREEnness (AGREE) method – this is an analytical calculator that greatly facilitates the calculation of the environmental friendliness and safety of the developed methodology, which shows the result of the analysis in the form of a certain pictogram, which takes into account the impact of each analytical operation on the result obtained, which is marked by a variation in color from ecological-green to dangerous-red [64].

Nowadays, the most modern valuable matrices such as modified green analytical procedure index (MOGAPI) [65] and Complementary Green Analytical Procedure Index (Complex MOGAPI) [66] are used to evaluate the greenness of analytical methods.

Table 2 presents the obtained «green» pictograms of the spectrophotometric methods of the studied analytes for reactions with dyes described in the scientific literature using the AGREE and MOGAPI tools. We understood the problems in the environmental safety of the analytical methods at the stage of planning our research, as we calculated the «greenness» score by the AGREE tool. The highest «greenness» score (0.76) was given to the method of the spectrophotometric determination of AML by reaction with crystal violet, which involved the use of acidic medium, potassium bromide and potassium bromate (Al-Mohsen Khdadad [57]). Such an excellent result can be explained by the time of the methodology (2022 year) and the use of modern approaches. The lowest «greenness» score (0.67) was obtained in the methods [52, 55, 56], which was due to the use of the toxic solvent chloroform. MOGAPI tool identified major problems such as extraction and use of toxic solvents. Figures 12, 13 show the obtained AGREE and MOGAPI scores the proposed methods. As can be seen from the pictograms on Fig. 12, the AGREE assessment scores for the methods for the determination of AML, NIT, NIF, LAML was 0.74 and 0.68 for NIM, LAC. It was worth noting that operations 7 and 10 were marked in yellow in all the methods, which indicates significant waste and the type of solvent. Parameter 11 (toxicity of the reagent) was marked in orange for NIM and LAC, due to the use of acetonitrile solvent. As can be seen from Fig. 13, the MOGAPI assessment scores for methods for the determination of AML, NIT, NIF, LAML was 0.72 and 0.70 for NIM, LAC, which was consistent with the results of the AGREE tool and indicates acceptable green analysis.

Table 8 The results of the quantitative determinations of CCBs in tablets

№	Found content in dosage forms, mg					
	AML 10 mg	LAC 2 mg	LAML 5 mg	NIF 20 mg	NIM 30 mg	NIT 10 mg
1	10.410	2.038	4.981	19.832	30.512	10.120
2	9.991	2.036	5.022	19.889	30.247	10.087
3	9.979	2.062	5.090	20.115	3.613	10.248
4	9.958	2.068	5.143	20.448	30.022	10.259
5	10.003	2.103	5.193	20.307	29.453	10.427
6	10.057	2.140	5.203	20.585	29.766	10.390
Mean	10.066	2.075	5.105	20.196	30.102	10.255
SD	0.172	0.040	0.091	0.303	0.445	0.137
RSD	1.705	1.942	1.780	1.503	1.479	1.339

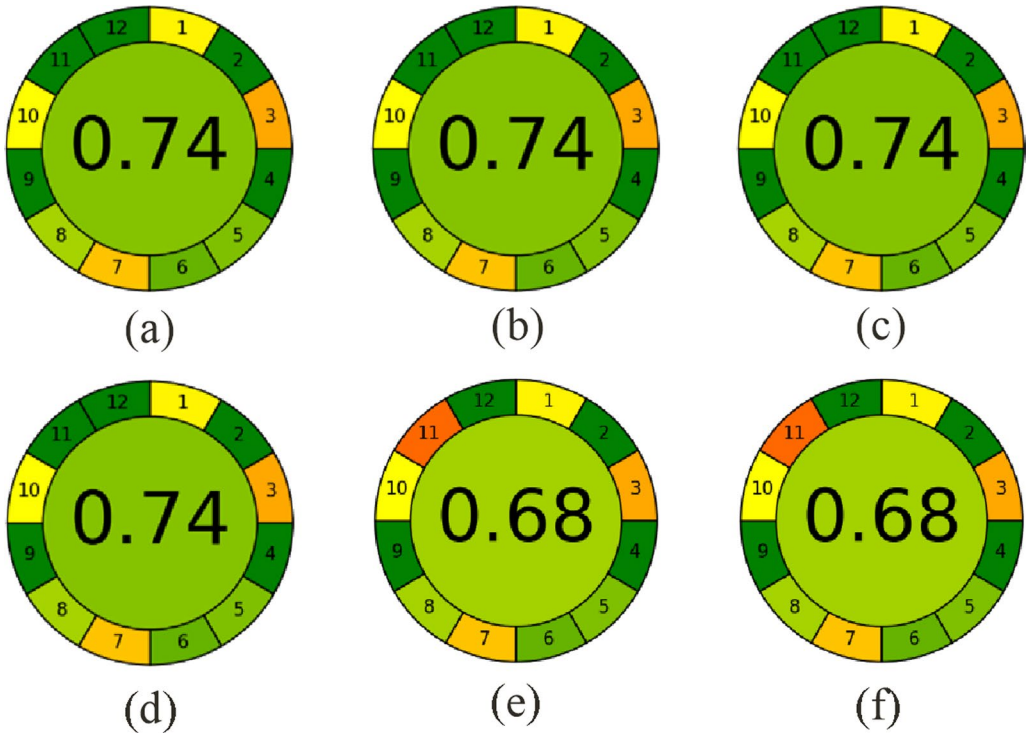


Fig. 12 AGREE assessment scores for the proposed methods used for the determination of AML (**a**), NIT (**b**), NIF (**c**), LAML (**d**), NIM (**e**), LAC (**f**)

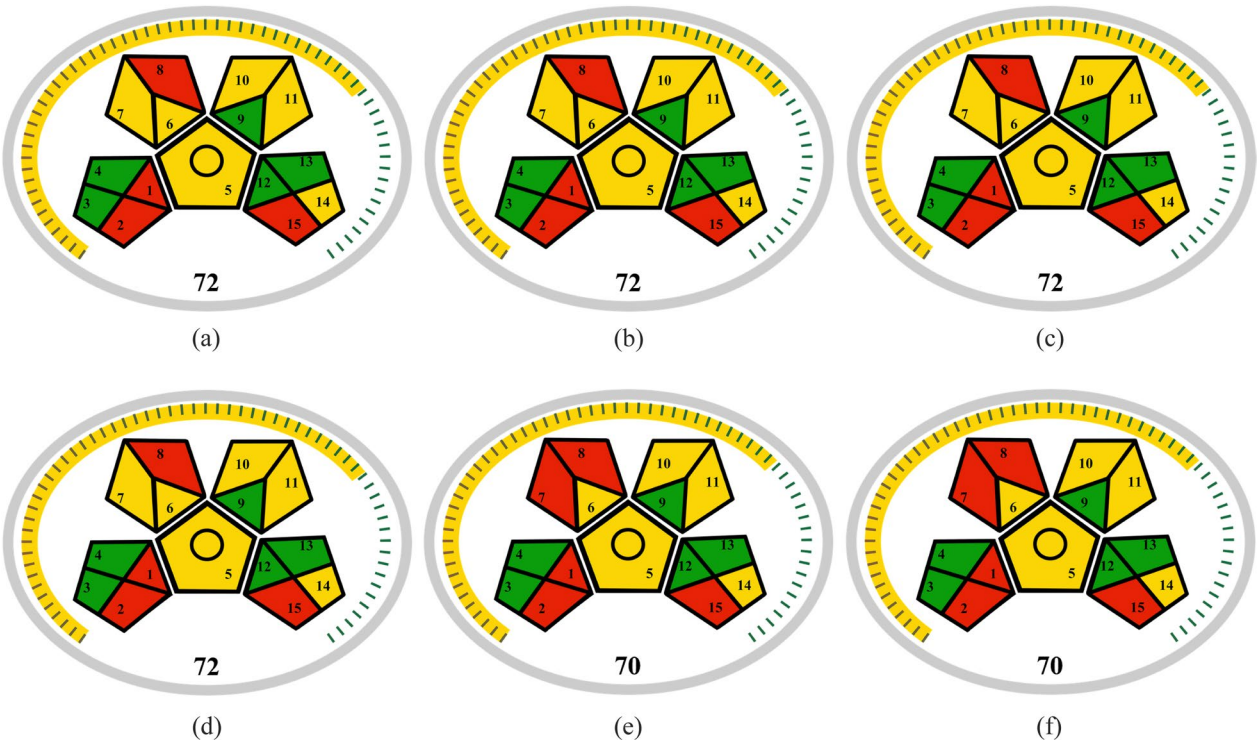


Fig. 13 MOGAPI assessment scores for the proposed methods used for the determination of AML (**a**), NIT (**b**), NIF (**c**), LAML (**d**), NIM (**e**), LAC (**f**)

Conclusion

In this work, thorough scientific research was carried out with the presentation of the method of selection of the optimal reagent, solvent, analytical wavelength and spectrophotometric methods for determining six CCBs were developed. In addition, the six studied CCBs were quantified using new «green», easy-to-implement, and cost-effective spectrophotometric approaches. The resulting ionic associates did not need to be extracted with organic solvents, which made the techniques safer for the environment. In addition, the proposed approach was more efficient in terms of time reliability, sensitivity and «greenness» than other recorded spectrophotometric methods. Moreover, the described approach can be easily implemented for routine pharmaceutical analysis.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13065-024-01378-x.

Additional file 1.

Author contributions

Horyn Mariana, Liubomyr Kryskiw, Tetyana Kucher, Nadiya Zarivna, Liliya Logoyda: Methodology and writing the original draft, Validation and reviewing, Horyn Mariana, Liubomyr Kryskiw, Tetyana Kucher, Nadiya Zarivna, Olha Poliak, Liliya Logoyda: Validation and reviewing, Liliya Logoyda: reviewing and publishing editing and supervision.

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Availability of data and materials

Data is provided within the manuscript or supplementary information files.

Declarations

Ethical approval and consent to participate

Not applicable.

Ethical Guidelines

Guidelines according to BMC journal.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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