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RESEARCH ARTICLE

Thin layer chromatographic compatibility study in preformulation of new transdermal therapeutic systems

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Objective: The compatibility of four binary active substances combinations adapalene – levofloxacin (ADP-LFX), adapalene – miconazole nitrate (ADP-MCZ), levofloxacin – meloxicam (LFX-MLX) and levofloxacin – miconazole nitrate (LFX-MCZ) was analysed to be comprised in new transdermal therapeutic systems. Also, the compatibility of selected active substances and four polymeric excipients (hydroxypropyl methylcellulose - HPMC 15000, hydroxypropyl methylcellulose - HPMC E5, ethyl cellulose - EC 10, and hydroxyethyl cellulose – HEC) was studied. **Methods**: Thin layer chromatographic method (TLC) and four selected mobile phases were used. On the plate (*in situ*) were obtained the binary combinations (active substances and active substance-polymer). **Results**: A good compatibility of ADP-LFX was found using ammonia : methanol : acetonitrile : methylene chloride 2:4:1:4 mobile phase. Using chloroform : acetone : glacial acetic acid 34:4:3 on the chromatogram of ADP-MCZ, only ADP spots appeared but without changes in the shape of the spots and R_f values. Any modifications of LFX and MLX spots (from LFX-MLX mixture) had been observed using toluene : glacial acetic acid : methanol 11:1:0.5 mobile phase, although LFX spots have remained on the baseline. Only LFX spots were visible from LFX-MLX and LFX-MCZ mixtures (ammonia : methanol : acetonitrile : methylene chloride 2:4:1:4 mobile phase). Distinctive spots were observed for ADP, LFX and MLX with variable results from no chemical interactions to limited chemical interactions when the compatibility with polymers was verified. **Conclusions**: ADP-LFX and LFX-MLX mixtures were found to be compatible. ADP with HPMC polymers and LFX with HPMC E5 and HEC had presented excellent compatibility; for the other binary combinations, different analytical methods will be necessary.

Keywords: TLC, adapalene, levofloxacin, meloxicam, miconazole nitrate

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Introduction

Chromatographic methods are used in preformulation studies, one of the simplest being thin layer chromatography (TLC) method. TLC is appropriate to determine the stability and compatibility of the compounds in a pharmaceutical formulation. The mixture of the selected substances will have to provide on the chromatogram identical spots with the individual compounds if there are no interactions between them [1].

In this paper, the compatibility between four active substances – adapalene (ADP), levofloxacin (LFX), meloxicam (MLX) and miconazole nitrate (MCZ) was pre-evaluated by TLC method, subject to the analysis four binary mixtures: ADP – LFX, ADP – MCZ, LFX – MLX, LFX – MCZ, as potential combinations in formulations of new transdermal therapeutic systems (TTSs). Nowadays, the development of new TTSs formula is increasing [2, 3]. Thereby, the use of binary mixtures of active substances became a new challenge in the pharmaceutical field (Figure 1).

In dermatology, the four selected active substances are used topically or systemically as valuable therapeutic compounds. The association of a retinoid (ADP) and a fluoroquinolone (LFX) pursuits the join of combining the anti-inflammatory and antibiotic effects, as the previous study was proving the efficiency of other similar combinations [4-6], LFX being active as well in topical applications [7-10]. The association of ADP with MCZ also could have therapeutic potential, MCZ being considered beneficial in the treatment of acne, both individually and in various combinations [11-13]. Co-administration of a fluoroquinolone (e.g. LFX) with a non-steroidal anti-inflammatory compound (MLX) or an antimycotic (MCZ) could be beneficial in complex therapy [14-16].

In addition, it has been studied the compatibility of selected active substances with a series of excipients as hydroxypropylmethylcellulose (HPMC) type E5 and 15000, ethyl cellulose type 10 (EC 10), and hydroxyethyl cellulose (HEC). These previously selected excipients as TTSs matrix-forming polymers have the advantage of forming gels in water from which flexible matrices can be obtained by evaporating the water to gentle heating.

Methods

Apparatus and reagents

A CAMAG chromatographic system (Camag, Switzerland) has been used: Nanomat 4 and capillary dispenser, dispenser magazine and capillary pipettes 2.0 μL, develop-

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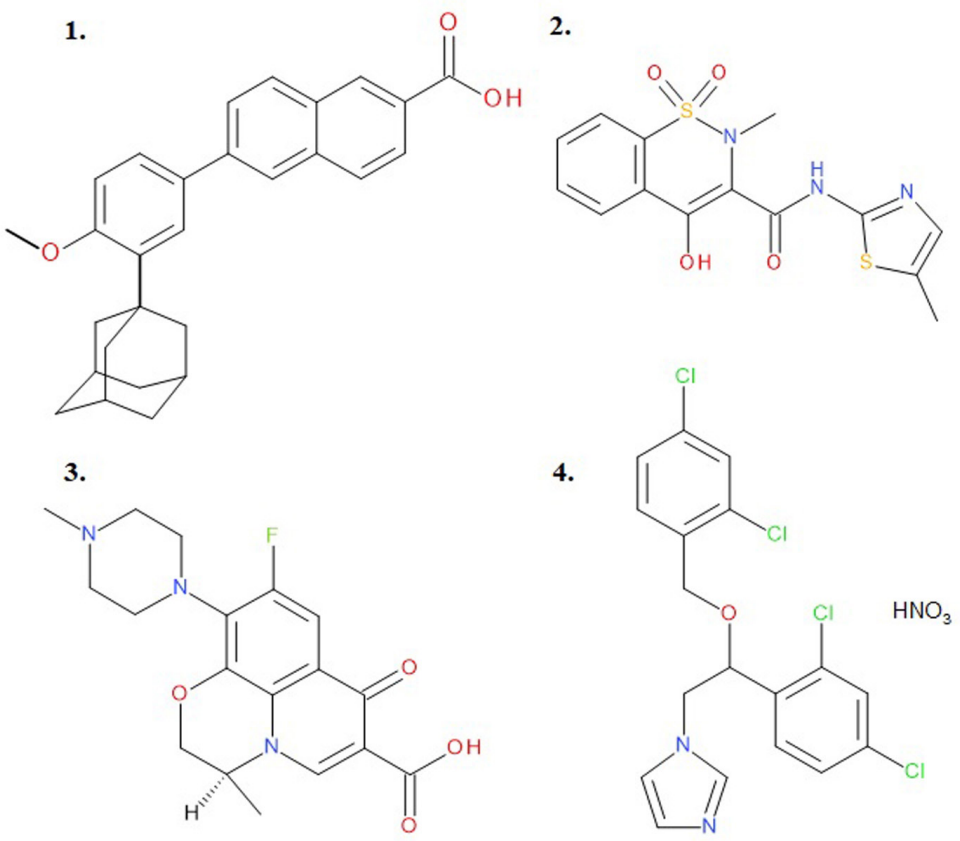

Fig. 1. The chemical structures of selected compounds: 1) ADP, 2) MLX, 3) LFX, and 4) MCZ

ing twin trough chamber for plates 20 x 20 cm, with glass lid, CAMAG dual wavelength UV lamp and a viewing box (two wavelengths, 254 and 366 nm). TLC silica gel 60 F_{254} and silica gel G (aluminium sheets 20 x 20 cm) (Merck, Germany) has been used as the stationary phase.

The used standard substances and reagents were obtained as follows ADP, LFX and MCZ from Sigma Aldrich (USA), and MLX from Techno Drugs & Intermediates Ltd (India); the other used solvents were acetonitrile, dioxane, methanol GR p.a. (Lach - Ner, Czech Republic), glacial acetic acid, acetone, methylene chloride (Chimopar Bucureşti, Romania), 25% ammonia (Microchim, Romania), chloroform (Chemical, Romania), toluene (Reactivul Bucureşti, România). Polymers were obtained as follows: HPMC E5 from Dow Chemical Co., Midland, USA, HPMC 15000 from Shin-Etsu Chemical Co, Ltd Tokyo, Japan, EC 10 and HEC from Sigma Aldrich Co., Germany.

Stock solutions were prepared in methanol (1 mg/mL concentration). The control solutions were obtained from stock solutions by dilution in an optimal ratio depending on the intensity of the preliminarily obtained spots.

Samples (2.0 µL) were applied using Nanomat 4 device. The distances between the spots were set at 15 mm, and the length of the edge of the TLC plate was set at 20 mm. After the TLC plates were dried at room temperature, were developed in a chamber previously saturated with mobile phase vapour for 30 min. The ascending mode at room temperature was used (22±2°C) until the solvent front reached 15 cm distance. Further, the plates were dried 15 min in air, and the spots were revealed using the dual-wavelength UV lamp (254/366 nm) or exposing the plate to iodine vapours.

Results

Selection of mobile phases. Several mobile phases have been tested, taking into consideration appropriate TLC methods for our compounds, LFX [17-19], MLX [20-22], and MCZ [23-24]. From the best of our knowledge, there is not any described method dealing with TLC determination, nor quantification of ADP in the scientific literature.

The selected mobile phases are presented in Table I.

The elution power of the mobile phases was calculated according to the following formula [25]:

$$\varepsilon^{\circ}_{\text{fază mobilă}} = (\%_{\text{solvent A}} \bullet \varepsilon^{\circ}_{\text{solvent A}})/100 + (\%_{\text{solvent B}} \bullet \varepsilon^{\circ}_{\text{solvent B}})/100 + \ldots (\%_{\text{solvent X}} \bullet \varepsilon^{\circ}_{\text{solvent X}})/100$$

The detection was noticed using UV light at 254 nm and 366 nm, as all selected compounds exhibited good adsorption, except MCZ (Table II).

The control solutions of two active substances were prepared and spotted separately on the baseline at 2 cm from the edge of the plate. Their binary mixture was obtained *in situ* on the baseline. In the same way, the spots were applied for each drug and the four polymers. The R_f values are presented in Table III. The retention parameter $\mathbf{R_M}$ was calculated, where $\mathbf{R_M = \log(1/R_f - 1)}$, which shows a linear relationship between the chromatographic and analytical

Table I. Mobile phases and their elution power values, and tested *in situ* binary mixtures (Ref. – references).

No.	Mobile phase composition and solvents ratio	ε°	Analysed binary mixtures (in situ)	Ref.
1	toluene : glacial acetic acid : methanol 11:1:0.5 (v/v/v)	0.37	LFX-MLX ADP-(polymer) MLX-(polymer)	[20]
2	chloroform : acetone : glacial acetic acid 34:4:3 (v/v/v)	0.46	ADP-MCZ LFX-MCZ	[23]
3	ammonia : methanol : acetonitrile : methylene chloride 2:4:1:4 (v/v/v/v)	0.74	ADP-LFX LFX-MCZ LFX-MLX LFX-(polymer)	[19]
4	ammonium acetate : dioxane: methanol 20:40:40 (v/v/v)	0.80	ADP-MCZ LFX-MCZ MCZ-(polymer)	[24]

Table II. Appearance of the active substances' spots at 254 și 366 nm.

Compound	Wavelength	
	254 nm	366 nm
MLX	purple-tinted	light blue with low luminescence
MCZ	very light purple, with low luminescence	-
LFX	purple	light blue with strong luminescence
ADP	purple	intense purple with strong luminescence

properties [25], respectively, the increased value of R_f are correlated with decreasing value of R_M (Table III).

Compatibility study between ADP and LFX. The selected no. 3 mobile phase was used. The chromatogram shows distinctive spots for control solutions and the binary mixture obtained *in situ* (Figure 2).

Compatibility between ADP and MCZ. For this purpose, the selected no. 2 mobile phase was used. The chromatogram shows distinctive spots only for ADP (obtained with the control solution and *in situ* mixture) with the same value of R_f (Figure 3, scheme b).

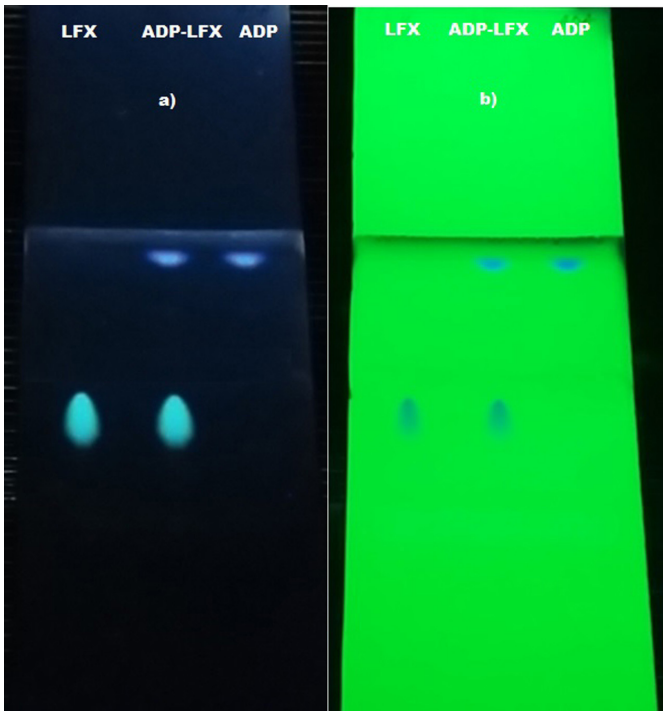

Fig 2. LFX, ADP-LFX, and ADP spots on the plate; wavelength a) 366 nm, b) 254 nm; no. 3 mobile phase

Table III. Obtained R_f values using the selected mobile phases.

Mobile phase	Active substance (from control solutions and mixtures obtained *in situ*)	R_f	R_M	Observations
No.1	MLX (control solution)	0.56	-0.10	
	MLX (from LFX-MLX)	0.56	-0.10	Same R_f value with control solution
	LFX (control solution)	-	-	Spot on the baseline
	LFX (from LFX-MLX)	-	-	Spot on the baseline
No. 2	MCZ (control solution)	-	-	No spot at both wavelengths
	MCZ (from LFX-MCZ)	-	-	No spot at both wavelengths
	LFX (control solution)	-	-	Spot on the baseline
	LFX (from LFX-MLX)	-	-	Spot on the baseline
	ADP (control solution)	0.95	-1.27	
	ADP (from ADP-MCZ)	0.95	-1.27	Same R_f value with control solution
No. 3	LFX (control solution)	0.62	-0.21	
	LFX (from LFX-MLX)	0.62	-0.21	Same R_f value with control solution
	LFX (from LFX-MCZ)	0.62	-0.21	Same R_f value with control solution
	ADP (control solution)	0.94	-1.19	
	ADP (from ADP-LFX)	0.94	-1.19	Same R_f value with control solution
	MLX (control solution)	-	-	No spot at both wavelengths
	MLX (from LFX-MLX)	-	-	No spot at both wavelengths

Compatibility between LFX and MLX. The chromatogram shows distinctive spots of LFX and MLX obtained with control solutions and *in situ* binary mixture at 254 nm; to note that the LFX spots have not migrated from the base line (Figure 4) using mobile phase no. 1.

Compatibility between LFX and MCZ. The chromatogram shows two distinctive spots only for LFX (from control solution and mixture) with the same value of R_f. Neither MLX or MCZ showed any spot at 366 nm (Figure 5) or 254 nm using mobile phase no. 3.

Compatibility of the active substances and polymers has been studied with the appropriate mobile phases, and the result has been comprised of Table IV. For MCZ, the results have been inconclusive with the mobile phase no. 2 (no visible spots on the plate at the two wavelengths). Thus, another mobile phase was tested: ammonium acetate R: dioxane: methanol 20:40:40 (v/v/v). Very pale spots at the reaction with iodine vapours and slight modification of MCZ R_f values were revealed.

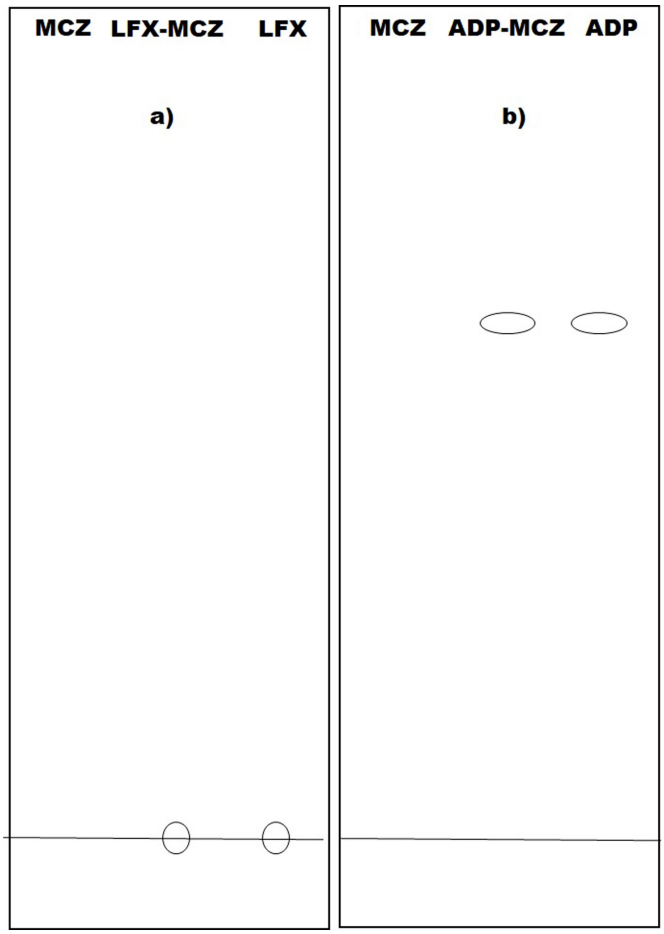

Fig. 3. Scheme of a) MCZ, LFX-MCZ, and MCZ spots on the plate; b) MCZ, ADP-MCZ, and MCZ spots on the plate; wavelength 254 nm; no. 2 mobile phase

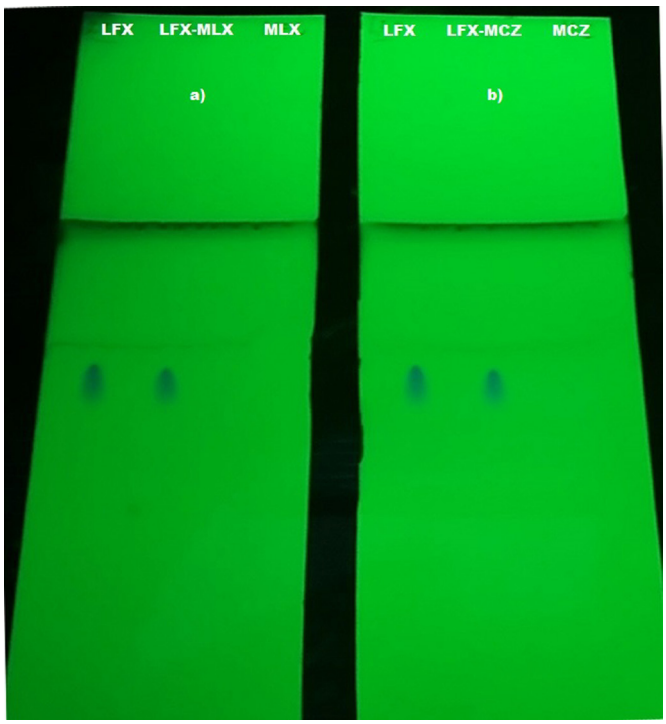

Fig. 5. a) LFX, LFX-MLX, and MLX spots on the plate; b) LFX, LFX-MCZ, and MCZ spots on the plate; wavelength 254 nm; no. 3 mobile phase

Fig. 4. MLX, LFX-MLX, and LFX spots on the plate; wavelength 366 nm; no.1 mobile phase

Compatibility of the active substances and polymers. The results of the compatibility study between the ADP, LFX and MLX are comprised in Table IV and Figure 6. Probably, due to the particular solubility of MCZ, the results were inconclusive regarding interactions with the other active substances using the mobile phase no. 2 and no. 4. In the no. 4 mobile phase, the obtained spots were very pale; small interactions with polymers occurred. The

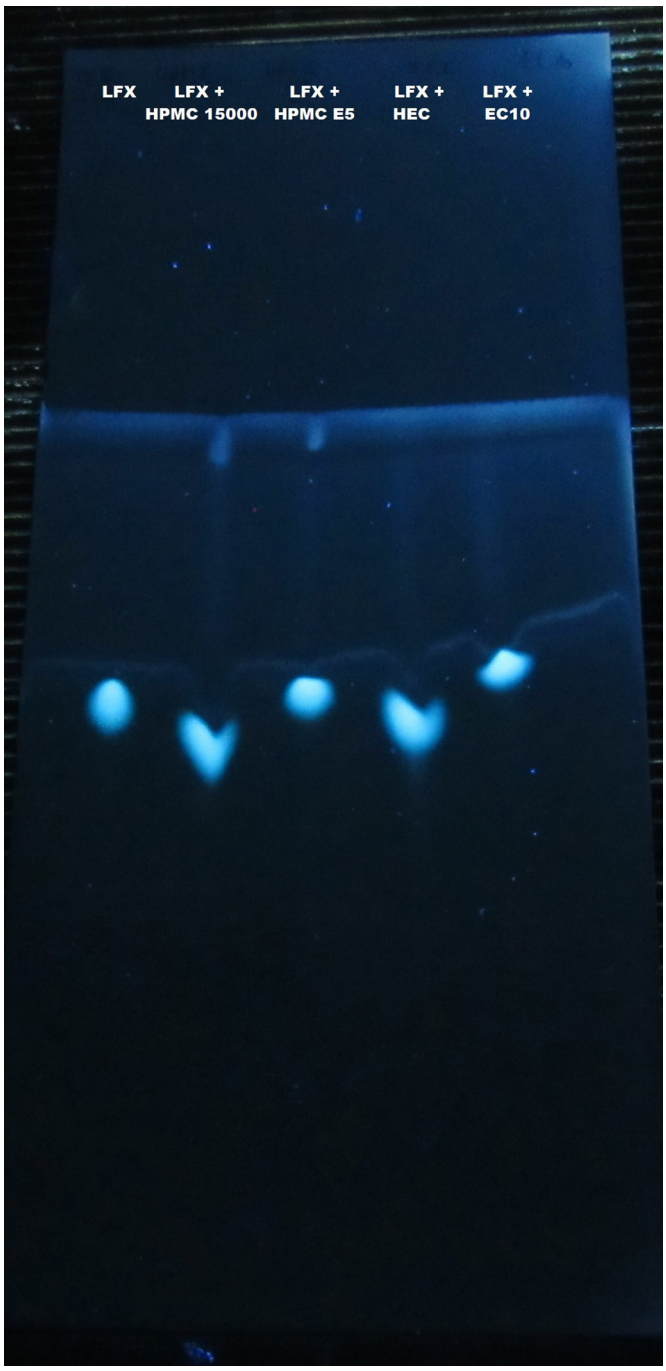

Fig. 6. LFX and in situ mixtures of LFX and polymers spots on the plate; wavelength 366 nm; no. 3 mobile phase

compatibility study of MCZ and selected polymers will be performed through other analytical methods.

Discussion

The analysis of obtained plates was carried out and discussed as follow:

Compatibility between ADP and LFX was tested through the TLC system using no. 3 mobile phase (Table III). This mobile phase has a 0.74 elution power and forces ADP to migrate to the top of the plate as a consequence of ADP solubility. ADP is soluble in polar aprotic solvents (tetrahydrofuran, dimethylsulphoxide and dimethylformamide), sparingly soluble in protic ethanol, and practically insoluble in water (polar protic solvents) [26, 27]. The no. 3 mobile phase contains acetonitrile, another polar aprotic solvent appropriate to be used on HPLC systems for ADP analysis [28-29]. ADP and LFX spots migrated with the mobile phase with different R_f values (Table III); it is clear that there is no incompatibility between the two compounds. Spots obtained from the mixture were identical to the controls and were visible at both wavelengths (Figure 2).

Though European Pharmacopoeia offers an identification method for MCZ, this was not appropriate for our compatibility experiment. Thus, no. 2 mobile phase was used (Table III) to study possible interactions between ADP and MCZ. The R_f value of ADP spot obtained from the mixture was identical to the R_f value of the control spot (R_f 0.95) (Figure 3) and could be viewed at both wavelengths. The MCZ spot was not visible at either of the two wavelengths but seemed to be clear that ADP spot has not been influenced. Apparently, no chemical interference occurred between the two compounds. In the next stage, it will be necessary to use other analysis methods to check the compatibility between the ADP and MCZ.

No. 1 mobile phase was used for testing the compatibility between LFX and MLX (Table III). The eluent was very appropriate for MLX (R_f 0.56). Instead, LFX spot has not migrated with the solvents and has remained on the baseline (Figure 4). On the no. 3 mobile phase the MLX spot was not visible, but the LFX spot has migrated with the mobile phase. The obtained R_f value of LFX spot from the mixture was the same with the R_f value of the control spot (R_f 0.62) (Figure 5). LFX spots were visible at both wavelengths. So, the two compounds seem to be compatible but will require other complementary methods to prove it.

Compatibility between LFX and MCZ was tested using no. 3 mobile phase (Table III). The obtained R_f value of LFX spot from the mixture was the same with the R_f value of the control spot (R_f 0.62) (Figure 5). LFX spots were visible at both wavelengths. So, the two compounds seem to be compatible, similar to the evaluation of compatibility between LFX and MLX.

The obtained results of the compatibility study of the four binary active substances and their binary combination with four selected polymers are comprised in Table V. The compatibility study of MCZ and selected polymers will be performed through other appropriate methods.

In the future, to collect the best compatibility data, more complementary methods will be necessary, such as spectroscopic methods and thermal analysis [30].

Conclusions

The obtained *in situ* ADP-LFX and LFX-MLX mixtures were found to be compatible. ADP-MCZ and LFX-MCZ mixtures require more specific analytical methods. ADP with HPMC polymers combinations and LFX with HPMC E5 and HEC combinations presented excellent compatibility by TLC method. Also, concomitant use of

Table IV. Results of the compatibility study between ADP, LFX and MLX and the four selected polymers (HPMC 15000, HPMC E5, HEC, and EC10).

Mobile phase	Compounds (from control solutions and mixtures obtained in situ)	R_f	R_M	Observations (comparisons to control solutions)
No. 1	ADP (control solution)	0.58	-0.14	
	ADP (from the mixture with HPMC 15000)	0.57	-0.12	a slight decrease in R_f values
	ADP (from the mixture with HPMC E5)	0.57	-0.12	
	ADP (from the mixture with HEC)	0.56	-0.10	
	ADP (from the mixture with EC10)	0.55	-0.08	
No. 3	LFX (control solution)	0.59	-0.15	
	LFX (from the mixture with HPMC 15000)	0.52	-0.03	a decrease in R_f value
	LFX (from the mixture with HPMC E5)	0.60	-0.17	
	LFX (from the mixture with HEC)	0.59	-0.15	same R_f value with control solution
	LFX (from the mixture with EC10)	0.63	-0.23	a slight increase in R_f value
No. 1	MLX (control solution)	0.57	-0.12	
	MLX (from the mixture with HPMC 15000)	0.51	-0.01	
	MLX (from the mixture with HPMC E5)	0.52	-0.03	
	MLX (from the mixture with HEC)	0.51	-0.01	
	MLX (from the mixture with EC10)	0.53	-0.05	

Table V. The result of the compatibility study; detection at 254 nm, 366 nm; *expose to iodine vapour

AS 1	AS 2 Mobile phase	ADP	LFX	MLX	MCZ	HPMC 15000	HPMC E5	HEC	EC 10
ADP	No. 1	x	x	x	x	MI	MI	MI	MI
LFX	No. 3	NI	x	NI	NI	SI	SI	MI	MI
MLX	No. 1	NI	NI	x	x	MI	MI	MI	MI
MCZ	No. 2	NI	NI	x	x	x	x	x	x
	No. 4 *	IC	IC	x	x	MI	MI	MI	MI

AS – Active substance, No interactions – NI, Minor interactions – MI, Strong interactions – SI, IC – Inconclusive Chromatogram, x – no determination

several spectroscopic and thermal methods will allow a better understanding of physicochemical drug-drug and drug-polymer interactions and will be helpful in the preformulation stage of TTSs.

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Authors' contribution

OLM (Data curation; Formal analysis; Investigation; Methodology; Visualization; Writing – original draft)
DNB (Data curation; Formal analysis; Methodology; Writing – original draft)
NT (Conceptualization; Methodology; Supervision; Validation; Writing – original draft)
AR (Conceptualization; Methodology; Project administration; Supervision; Validation; Writing – review & editing)

Conflict of interest

None to declare.

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