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Research Article

COMPARISON OF DIFFERENT WAYS OF CHROMATOGRAPHIC SPOTS VISUALIZATION IN DETERMINATION OF ANTYHISTAMINE SUBSTANCES

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ABSTRACT
Importance of compounds investigated (loratadine, desloratadine, cetirizine dihydrochloride, levocetirizinedihydrochloride) in contemporary pharmacy causes necessity of finding the fast and easy method for their identification. The aim of this work is to find the best way of visualization of chromatographic spots in qualitative determination of compounds investigated during analysis by thin layer chromatography. The standard solutions of compounds investigated were prepared in ethanol or methanol. The different mobile phases for particular compounds were used. There were also used many ways of spot visualization i.e. exposure to UV light, dipping in solution of visualizing agents, visualization in iodine vapour and photo catalytic visualization. The best visualization effect gave exposure to UV light and dipping in solution of bromophenol blue in the case of loratadine and desloratadine, respectively; and visualization in iodine vapour in the case of cetirizine and levocetirizine. Using photo catalytic degradation had no use in the case all of compounds investigated.
Keywords: Antihistamine, Thin-layer chromatography, Visualization

INTRODUCTION

The subject of work are some compounds with antihistamine activity i.e. cetirizine dihydrochloride, levocetirizinedihydrochloride, loratadine and desloratadine. All of them exist in market as components of pharmaceutical preparations produced by many different producers. It must be underlined that levocetirizinedihydrochloride is active enantiomer of cetirizine dihydrochloride and desloratadine is active metabolite of loratadine[1]. The method of analysis used at this work is thin layer chromatography – easy and fast method of qualitative analysis. The antihistaminic preparations are widespread nowadays, that is why the fast method of their identification could be very useful. The aim of this work is comparison of different ways of chromatographic spot visualization in qualitative determination of cetirizine dihydrochloride, levocetirizinedihydrochloride, loratadine and desloratadine. There are many works about using the visualizing agents for many different compounds[2-8]. The novelty of this work is using not only solutions of visualizing agent, iodide vapour or simply UV light but also using the reaction of photo catalytic degradation causing that chromatographic spot should be more visible. That reaction was successfully used previously to determine many classes of chemical compounds with biological activity[9], but there were no compounds such as cetirizine dihydrochloride, levocetirizinedihydrochloride, loratadine and desloratadine.

MATERIALS AND METHODS

The substance investigated were loratadine, desloratadine, cetirizine dihydrochloride and levocetirizinedihydrochloride, all supplied by Sigma-Aldrich, USA. The standard solutions of loratadine and desloratadine were prepared in 96 % ethanol (POCh, Poland) whilst the standard solution of cetirizine dihydrochloride and levocetirizinedihydrochloride were prepared in methanol (POCh, Poland). The initial concentration of all solutions was 1 mg/1 mL. The chromatographic development was carried out on plates precoated with silica gel $60F_{254}$ (#105554, Merck, Germany). The mixture of chloroform, ethyl acetate and acetone in volume ratio 15:15:21 was used as mobile phase in the case of loratadine analysis and mixture of ethyl acetate, n-butanol, 25 % ammonia and methanol in volume ratio 30:7:6:7 was used as mobile phase in the case of desloratadine analysis. The mobile phases for analysis of cetirizine dihydrochloride and levocetirizinedihydrochloride were acetonitrile – water – 25 % ammonia (44:5:1, v/v/v) and ethyl acetate – methanol – 25 % ammonia (36:10:6, v/v/v), respectively. All solutions, components of mobile phases were supplied by POCh, Poland. Chromatographic plates used were previously activated during 30 minutes in temperature of 120°C in the case of loratadine and desloratadine analysis. In the case of cetirizine dihydrochloride and levocetirizinedihydrochloride, plates were previously prewashed in methanol and then dried in 50°C during 10 minutes and dried in 110°C during 5 minutes for cetirizine and levocetirizine, respectively. Plates were developed in a room temperature, to the height of 7.5 cm, in glass chamber, previously saturated with mobile phase. The solution was spotted by use of micro syringe (Hamilton, USA). The maximum amount spotted was 10 µL. The ways of visualization of chromatographic spots were: exposure to UV light, iodine vapour, dipping in 5 % water solutions of the following visualizing agents: methyl green (Fluka, USA), methyl violet, gentian violet, phenol red and brilliant-cresol blue (POCh, Poland), alkaline blue and bromophenol blue (Merck, Germany) and cresol red (Eurochem BGP, Poland) - all result of visualization were observed without and in UV light, and also photocatalytic visualization was used i.e. dipping in solutions of mixture of 0.1M $KMnO_4$, 0.1M KI, 0.1M KBr or 0.1M KCl with TiO_2 and then exposure to light which $\lambda = 366$ nm; all compounds ($KMnO_4$, KI, KBr, KCl) were supplied by POCh, Poland, whilst TiO_2 by Evonik Industries, Canada. All

chromatographic plates were evaluated twice – without and in UV light.

RESULTS AND DISCUSSION

As a result of observation of chromatographic plates after development and visualization, the limit of detection of loratadine, desloratadine, cetirizine dihydrochloride and levocetirizinedihydrochloride were determined. The smallest amount of substance investigated (limit of detection) in μg/spot are presented in Table 1. The smallest visible amount was observed in UV light for loratadine (0.06 μg/spot) and desloratadine (0.04 μg/spot). The similar limit of detection (LOD) was obtained with use of solution of alkaline blue and cresol red (both 0.05 μg/spot) as well as bromophenol blue (0.04 μg/spot) for desloratadine and using the iodine vapour (0.05 μg/spot) in the case of levocetirizinedihydrochloride. In the case of cetirizine dihydrochloride the best results of LOD was obtained by visualization in iodine vapour (0.2 μg/spot). One of the methods of visualization used was photo catalytic reactions. The TiO_2 which shows properties of semiconductor in mixture with $KMnO_4$, KI, KBr and KCl was used for visualization. The semiconductor can be used because its surface electrons are excited by the absorptions of light radiation[10]. Then transition of the electron from the valence band to the conduction band is observed and electron (e^-) – hole (h^+) pairs are created[11]. The electron holes can oxidize iodide, chloride and bromide and halide radicals can be created[12]. These created radicals can react with compounds on the chromatographic plates causing better visibility of chromatographic spots[9]. Using photo catalytic degradation for spots visualization the mixtures of $KMnO_4$ and TiO_2 was useless in the case of loratadine and cetirizine dihydrochloride, whilst in the case of desloratadine and levocetirizinedihydrochloride the results obtained were much worse than the best results for these compounds (0.2 and 0.4 μg/spot for cetirizine and levocetirizine, respectively). The mixture of KI and TiO_2 turned out absolutely useless for all of compounds investigated. In the case of determination of loratadine the mixtures of KBr or KCl with TiO_2 used for visualization gave worse results that the other of methods of visualization analysed. In the case of desloratadine the smallest amount possible to determine is 0.1 μg/spot but it is not the best result obtained in relation to the other method of visualization. The mixtures of KBr or KCl with TiO_2 were completely useless in determination of cetirizine dihydrochloride and levocetirizinedihydrochloride. It should be emphasized that the chromatographic spots were only visible after drying in the air and in UV light. Moreover the using photocatalytic degradation caused worse visualizing effects for both loratadine and desloratadine in relation to visualization only in UV light.

Table 1: The Limit of Detection of Loratadine, Desloratadine, Cetirizine Dihydrochloride and Levocetirizine Dihydrochloride

Way of visualization	Loratadine [μg/spot]		Desloratadine [μg/spot]		Cetirizine dihydrochloride [μg/spot]		Levocetirizinedihydrochloride [μg/spot]	
	without UV	in UV	without UV	in UV	without UV	in UV	without UV	in UV
UV light	-	0.06	-	0.04	-	4	-	0.4
methyl green	0.1	0.1	-	0.3	5	-	0.6	0.6
methyl violet	-	0.1	-	0.1	-	-	0.1	-
gentian violet	0.4	0.3	0.3	0.3	2	2	0.4	0.2
phenol red	0.4	0.2	-	0.2	3	3	0.6	0.6
brilliant-cresol blue	-	0.3	-	0.2	3	3	0.2	0.2
alkaline blue	0.3	0.3	-	0.05	-	-	0.8	-
bromophenol blue	0.4	0.2	0.1	0.04	3	3	0.6	0.6
cresol red	0.3	0.1	-	0.05	2	2	0.6	0.6
$KMnO_4 + TiO_2$	-	-	0.2	0.2	-	-	0.4	0.4
$KI + TiO_2$	-	-	-	-	-	-	-	-
$KBr + TiO_2$	-	0.5	-	0.1	-	-	-	-
$KCl + TiO_2$	-	0.4	-	0.1	-	-	-	-
iodine vapour	-	-	0.2	0.2	-	0.2	0.05	0.05

CONCLUSION

It turned out that the best visualizing effects for loratadine and desloratadine were when using only UV light. The amount possible to see was 0.06 μg/spot (loratadine) and 0.04 μg/spot (desloratadine), but in the case of desloratadine the same effect gave the dipping in solution of bromophenol blue. The best visualizing effects for cetirizine dihydrochloride and levocetirizinedihydrochloride were obtained for visualization in iodine vapour - 0.2 μg/spot for cetirizine and 0.05 μg/spot for levocetirizinedihydrochloride. Moreover the using photo catalytic degradation for spots visualization had no use in the case all of compounds investigated.

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