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Extraction of organic acids by ion-pair extraction with tri-*n*-octylamine — VIII. Identification of synthetic dyes in pharmaceutical preparations

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Abstract: Synthetic dyes were extracted from syrups, oral suspensions, tablets, gelatin capsules, suppositories and granules by ion-pair formation with tri-*n*-octylamine (TnOA) and back-extracted with perchlorate ions. Identification was performed by TLC on cellulose layers and by reversed phase ion-pair HPLC.

Keywords: *Synthetic dyes; pharmaceutical preparations; ion-pair extraction; identification.*

Introduction

The routine control of pharmaceuticals requires not only the qualitative and quantitative analysis of the active constituents, but also the identification of all the excipients. Many commercially available pharmaceuticals for oral use contain one or several colorants. In the past, synthetic dyes have been identified by paper chromatography (PC) [1–3], thin-layer chromatography (TLC) [4–6] or high performance liquid chromatography (HPLC) [7].

Various sample preparation methods were used before the chromatographic analysis, including adsorption on wool yarn [1, 2], aluminum oxide [3] or polyamide [4], and solvent [6] or ion-pair [5] extraction. The applicability of ion-pair extraction with tri-*n*-octylamine (TnOA) to the analysis of synthetic dyes in pharmaceutical preparations is described in this paper. The extraction procedure has been successfully applied to the quantitative analysis of synthetic colorants in various foodstuffs [8–10]. Identification was performed by TLC and HPLC as previously described [8–11].

Experimental

Apparatus

An Orion Ionalyser 601 with a combined glass electrode was used to measure pH values. The chromatograph was a Varian LC 5060 equipped with a standard UV 254 nm detector as well as a Varian Varichrom® variable wavelength detector. Integrations were

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performed with a Varian Vista CDS 401. Injections were made with a Rheodyne 7125 injector (loop size: 10 μl).

Chromatographic supports

For HPLC, 250 × 4 mm i.d. column packed with 10-μm LiChrosorb RP-18 (Merck, Darmstadt, FRG) was used.

For TLC, 20 × 20 cm cellulose pre-coated thin-layer plates (Merck), layer thickness 0.1 mm, were used.

Reagents

Tri-*n*-octylamine was obtained from Aldrich Europe (Beerse, Belgium). Tetrabutylammonium phosphate (TBA) was purchased as a 0.5 M aqueous solution (Altex). Polyamide (for chromatography) was obtained from Woelm (Eschwege, FRG). All other reagents used were analytical grade products from Merck, except methanol which was HPLC grade. Phase separating filters (silicone treated) were from Whatman.

Buffers

The compositions for 2 l. volumes having an ionic strength of 0.1 were as follows:
pH = 4.0:3.20 ml H_3PO_4 1 M and 27.80 g $NaH_2PO_4.H_2O$.
pH = 5.5:24.65 g $NaH_2PO_4.H_2O$ and 1.86 g $Na_2HPO_4.2H_2O$.
pH = 7.0:3.77 g $NaH_2PO_4.H_2O$ and 9.38 g $Na_2HPO_4.2H_2O$.
Solutions were made in double distilled water.

Mobile phases

HPLC mobile phases were composed of a mixture of methanol and phosphate buffer (pH 7.0) to which was added 5 mM TBA. All eluents for HPLC were filtered through a 0.45 μm filter (Millipore).

For TLC, ethylacetate–*n*-propanol–ammonia–water (35:35:20:20, v/v/v/v) was used.

Extractions

Syrups. An aliquot of 5 ml of syrup was transferred to a glass, screw-capped, 30 ml centrifuge tube, followed by 5 ml of phosphate buffer (pH 5.5) and 5 ml of a 0.1 M TnOA solution in chloroform. The tube was shaken for 30 min and then centrifuged (15 min at 2000 rpm). The supernatant was discarded and the organic phase was filtered through a phase-separating filter. An aliquot of 3 ml of the filtrate was transferred to a second (glass, screw-capped, 10 ml) centrifuge tube and was extracted with 3 ml of a 0.1 M sodium perchlorate solution in water. The perchlorate extract was then used for analysis (TLC and HPLC).

Suspensions. The powder (for suspension) was first suspended in the prescribed volume of water. An aliquot of 5 ml of the thoroughly shaken suspension was transferred to a glass, screw-capped, centrifuge tube and the procedure followed was then the same as described for syrups, starting from "5 ml of phosphate buffer (pH 5.5) . . ."

Tablets. Two to three tablets were crushed in a mortar and the powder obtained was dissolved as completely as possible in *ca* 50 ml of distilled water. An aliquot of 5 ml of this suspension was used for analysis following the procedure described for syrups, starting from "5 ml of phosphate buffer (pH 5.5) . . ."

Coated tablets. Two to three coated tablets were wetted with 10 ml of distilled water until complete dissolution of the coloured coating and then 5 ml of this solution was used for the analysis. The procedure followed was the same as for syrups starting from "5 ml of phosphate buffer (pH 5.5) . . ."

Gelatin capsules. One to three gelatin capsules were opened, emptied, and the capsules dissolved in *ca* 20 ml of warm (*ca* 50°C) distilled water. An aliquot of 5 ml of this solution was transferred to a glass centrifuge tube, and 10 ml of phosphate buffer (pH 4.0) and *ca* 1 g of polyamide powder were added. The tube was shaken for 15 min then centrifuged for 5 min at 2000 rpm and the supernatant discarded. The polyamide was washed three times with 10 ml of warm distilled water (shaking time: 5 min, centrifugation: 5 min at 2000 rpm) and the dyes were desorbed with 10 ml of a methanol–ammonia (95:5 v/v) mixture (shaking time: 5 min, centrifugation: 5 min at 2000 rpm). The methanol–ammonia extract was evaporated under a stream of nitrogen and the residue was redissolved in 10 ml of phosphate buffer (pH 5.5). The procedure was then the same as for syrups, starting from "5 ml of a 0.1 M TnOA solution in chloroform . . ."

Suppositories. One suppository was melted in 25 ml of warm (*ca* 50°C) phosphate buffer (pH 5.5), and after cooling down the suspension, 10 ml of the coloured aqueous solution was used for analysis as described for syrups, starting from "5 ml of a 0.1 M TnOA solution".

Granules. Approximately 10 g of the granules were crushed in a mortar and 250 mg of this powder dissolved as completely as possible in 20 ml of phosphate buffer (pH 5.5) by shaking it for 10 min in a glass, screw-capped, centrifuge tube. The procedure followed was then the same as described for syrups, starting from "5 ml of a 0.1 M TnOA solution . . .".

Results and Discussion

The pharmaceuticals analysed were selected in such a way that as many different dyes as possible were obtained, with the emphasis on samples which contained more than one colorant.

Syrups

The procedure used for syrups was very similar to the one used previously for the analysis of alcoholic beverages [8] and soft drinks [12]. As described in those papers, it was possible to identify (by TLC) dyes extracted as ion-pairs with TnOA by comparing their R_f value with the value obtained for reference dyes spotted as aqueous solutions. This was especially the case when polar mobile phases were used on cellulose layers [11]. The TLC identification of dyes extracted from syrups was therefore attempted directly in the chloroform phase. However, in most cases, a different elution pattern was observed for the free dyes and dyes spotted as ion-pairs. This deviation is probably due to the presence of co-extracted compounds or to the presence of some pairing ion. In consequence, dyes were extracted back to an aqueous phase by a displacement with perchlorate ions. Earlier results [8] showed that erythrosine was very badly extracted with 0.1 M sodium perchlorate. Therefore, in the case of samples which contained erythrosine, the back-extraction was performed with a 0.1 M perchlorate solution in 0.01

M sodium hydroxide. By this means a quantitative back-extraction was obtained. However, it should be noted that these alkaline extracts cannot be stored for longer than 1–2 h because of the discoloration of erythrosine in this medium. Upon neutralization with 0.01 M hydrochloric acid this extract can be stored for a much longer period.

The results listed in Tables 1 and 2 (for TLC) and Table 3 (for HPLC) indicate that all dyes were successfully identified by the procedure used. However, in Folixin® and Cotrane®, E131 and E110 respectively, were not detected. This was due to the very low concentration of these dyes in those samples. The identification was nevertheless successful when the extraction was repeated in a separation vessel using 25 ml of syrup, 50 ml of phosphate buffer (pH 5.5) and 5 ml of 0.1 M TnOA in chloroform.

Table 1
R_f values of dye standards ($n = 5$)

Dye	R_f (mean ± S.D.)
Tartrazine (E102)	0.27 ± 0.01
Sunset Yellow (E110)	0.75 ± 0.02
Azorubine (E122)	0.18 ± 0.01
Amaranth (E123)	0.35 ± 0.01
Cochenille red A (E124)	0.54 ± 0.01
Erythrosine (E127)	0.85 ± 0.02
Patent blue V (E131)	0.93 ± 0.02
Indigotin (E132)	0.40 ± 0.01
Brillant Black (E151)	0.11 ± 0.01

The HPLC eluents were monitored simultaneously at 254 nm and at the wavelength of maximal absorbance (in the visible part of the spectrum). The chromatograms obtained with UV detection contained several other peaks, which were due to the drug(s) or to other excipients such as preservatives and saccharin, which were also extracted by this technique [12]. These other peaks overlapped in some cases the dye peak and hence complicated the identification. However, chromatograms monitored at the wavelength of maximal absorbance of the dye displayed only the dye peak(s), as shown in Fig. 1. As a consequence, the eluent was always monitored at the wavelength of maximal absorbance of the expected compound. In cases where more than one dye was expected, an intermediate wavelength was selected.

Suspensions, tablets and coated tablets

As shown in Tables 2 and 3, the identification was successful for all samples, both in TLC and HPLC. In HPLC, only data obtained at the wavelength of maximal absorbance are presented. It was observed that for samples which contained more than one dye, the R_f values of the individual dyes were somewhat lower than for dyes chromatographed separately. This was mainly due to an interaction between the dyes and not to matrix constituents since the same phenomenon was also observed for pure dye mixtures.

Gelatin capsules

Gelatin is a protein which strongly adsorbs colorants. This strong interaction prevents the ion-pair formation with TnOA [10]. In the past we have overcome this difficulty by adsorbing the dye first on polyamide and washing away the gelatin [10]. As this

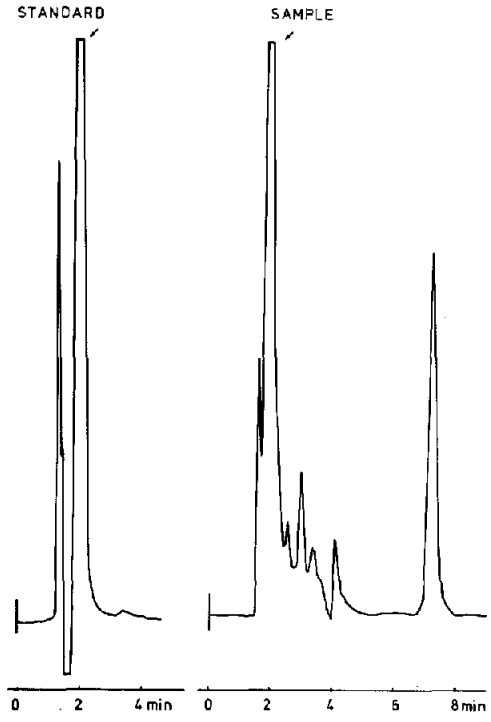

FIG. 1 A

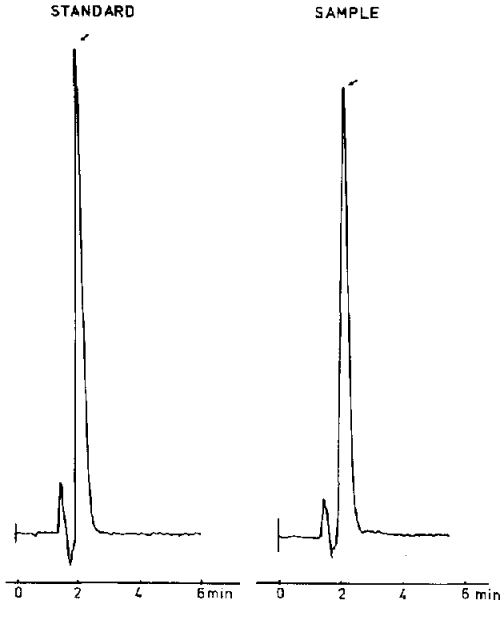

FIG. 1 B

Figure 1
Chromatogram of Tartrazine (E102) Standard and Diclocil® extract. Mobile phase 0.005 M TBA in methanol–phosphate buffer pH: 7.0(60:40, v/v). Flowrate: 1.5 ml min^{-1}. (A) Detection at 254 nm; (B) detection at 430 nm. Arrow indicates elution peak of tartrazine.

Table 2
R_f values of dyes extracted from pharmaceuticals

Name of brand	Label claim	R_f value found
Syrups		
Folixin	E123 + E131	0.35 + 0.91
Achromycine	E123	0.35
Sigmamycine	E127	0.85
Ledermycine	E123	0.36
Vibramycine	E127	0.84
Rondomycine	E110	0.74
Cotrane	E124 + E110	0.53 + 0.74
Codeigne	E102	0.27
Tussionex	E102	0.26
Biocodone	E102	0.27
Suspensions		
Ilotycine	E123	0.35
Diclocil	E102	0.26
Penstapho	E127	0.84
Pentrexyl	E123	0.36
Minikel	E102 + E124	0.24 + 0.52
Vithathion	E102 + E124	0.24 + 0.53
Tablets		
Presinol	E102	0.26
Peritrine	E102 + E132	0.25 + 0.39
Sinaxar	E102 + E122	0.25 + 0.18
Percotène	E123 + E132	0.34 + 0.39
Thiamalgyl	E110	0.75
Meratonic	E127	0.84
Piptal	E102 + E110	0.26 + 0.75
Flagyl	E102	0.27
Galatur	E123	0.34
Pentabs 500	E102	0.26
Aldactone-A	E102 + E123	0.26 + 0.33
Coated tablets		
Salicylamide	E127	0.80
Sedapersantine	E132	0.39
Octometine	E131	0.90
Trenteron	E110	0.72
Neodistilbene	E132	0.37
Parmanil	E102 + E122 + E151	0.24 + 0.15 + 0.08
Difrarel	E102 + E132	0.24 + 0.36
Ferronicum	E124 + E102	0.52 + 0.23
Gelatin capsules		
Biphetamine-T	E102 + E132 + E123	0.24 + 0.32 + 0.38
Doridène	E132	0.39
Macline	E132 + E123 + E127 + E102	0.39 + 0.33 + 0.81 + 0.25
Cillimycine	E123 + E151	0.34 + 0.10
Dytac	E102 + E123	0.26 + 0.34
Tetralysol	E102 + E127	0.26 + 0.82
Penbritin (8 caps.)	E123 + E102 + E127 + E151	0.34 + 0.26 + 0.82 + 0.10
Penbritin (16 caps.)	E123 + E102 + E127 + E132	0.35 + 0.26 + 0.82 + 0.40
Quadracycline	E127 + E132 + E102	0.83 + 0.40 + 0.25
Suppositories		
Sedolsuppo	E110	0.73
Cedilanide	E110	0.72
Granules		
Normacol	E151 + E124 + E110	0.10 + 0.52 + 0.73

Table 3
Retention times of standard dyes and dyes extracted from pharmaceuticals. Chromatograms were monitored at the wavelength of maximal absorbance.* Flow rate: 1.5 ml min^{-1}

Brand name	Dye	MeOH (%)	Retention time sample (standard)
Syrups			
Achromycine	E123	60	3.0 (3.1)
Sigmamycine	E127	40	4.1 (4.3)
Rondomycine	E110	50	2.0 (2.0)
Tussionex	E102	63	2.3 (2.4)
Folixin	E123	60	2.9 (3.1)
	E131	40	5.4 (5.5)
Suspensions			
Diclocil	E102	60	2.9 (3.0)
Pentrexyl	E123	60	4.7 (4.8)
Penstapho	E127	40	4.5 (4.3)
Minikel	E102 + E124	60	2.9 + 11.7 (3.0 + 11.8)
Coated tablets			
Octometine	E131	40	5.6 (5.7)
Neodistilbene	E132	62	3.1 (3.1)
Difrarel	E102 + E132	63	4.8 + 6.6 (4.8 + 6.7)
Granules			
Normacol	E110 + E124 + E151	57	4.6 + 7.4 + 9.0 (4.8 + 7.1 + 9.1)

* Absorption maxima: E102, 430 nm; E104, 415 nm; E110, 490 nm; E122, 515 nm; E123, 520 nm; E124, 515 nm; E127, 530 nm; E131, 620 nm; E132, 610 nm; E142, 635 nm; E151, 570 nm.

procedure was successful for the analysis of gelatin-containing sweets, it was also employed here. The results listed in Tables 1 and 2 indicate that this method is also applicable to gelatin capsules.

Suppositories and granules

The colorant E110 was identified successfully by TLC in both brands of suppositories. No significant interference was observed from the suppository base. The identity of the dyes which were extracted from the granules was confirmed both by TLC and HPLC. Since it was found that the extracts contained a very high amount of dyes, the areas of the peaks were calculated and a quantitation was performed versus calibration graphs (0.2–6.0 mg/100 ml of each dye). Chromatograms were recorded at 530 nm. The following results were obtained for the granules: 952.5 ± 55.2 mg kg^{-1} of E110, 287.3 ± 25.0 mg kg^{-1} of E124 and 90.5 ± 7.2 mg kg^{-1} of E151.

Conclusion

The results indicate that synthetic dyes can be extracted from pharmaceutical preparations by means of an ion-pair formation with tri-*n*-octylamine. The schemes developed previously [8–10] were generally applicable and only a few minor additional steps were required.

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