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Analysis of phenols in pharmaceuticals by liquid chromatography after pre-column labelling and on-line post-column photochemical derivatization

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Abstract

Reversed phase high-performance liquid chromatographic (RP-HPLC) methods are proposed for the analysis of phenol, thymol, chlorocresol and chloroxylenol in commercial pharmaceutical dosage forms. The use of 1-fluoro-2,4-dinitrobenzene as UV labelling reagent in pre-column derivatization has been investigated. The compound reacts rapidly (2 min) under mild conditions (ambient temperature or 40°C) with phenols to give ethers that can be separated by RP-HPLC and detected at 292 nm. The other procedure involves a post-column on-line photochemical conversion. A photoreactor was arranged between the analytical column and the fluorescence ($\lambda_{ex} = 270$ nm, $\lambda_{em} = 310$ nm) and UV-diode array ($\lambda = 270$ nm) detector to enhance the performance of the methods. Additional informations of the analyte structure and photoreactivity by UV spectra (photoreactor 'on' and 'off') were obtained. The methods showed good selectivity and sensitivity.

Keywords: RP-HPLC; 1-Fluoro-2,4-dinitrobenzene; Photochemical derivatization; Phenols

1. Introduction

Phenols and chlorophenols are antiseptics with antibacterial and antifungal activity [1] that are widely used in pharmaceuticals and cosmetics to protect the health of the consumer, as well as to maintain the potency and stability of the product formulations. The analysis of preservatives often presents difficulties largely due to the low levels present in commercial preparations and the complexity of the sample matrices [2–6]. The derivatization technique presents two general goals: to increase detection sensitivity, normally introducing suitable chromophores or fluorophores or obtaining a different compound with higher response, and to increase the selectivity by applying a specific and selective derivatization reaction to derive only the compound or compounds of interest and to detect them selectively in a complex matrix. Derivatization reactions can be applied either before or after the chromatographic separation. Most derivatization reactions consist of the addition of one or more chemical reagents to the analyte in order to transform it into a more detectable one. When the light is used instead of the reagent, the procedure is called photochemical reaction and normally they are simple, flexible and clean [7].

In previous researches, we focused on 2-chloro-6, 7-dimethoxy-3-quinolinecarboxaldeide as a selective fluorescent labelling for chlorophenols and successfully applied to determine by liquid chromatography (LC) chlorocresol and chloroxylenol present at very

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low concentrations (0.1–0.4%) as preservatives in pharmaceuticals [8]. As extension of these studies, the present work was aimed to evaluate alternative derivatization methods in LC for the analysis of phenols (chlorocresol, chloroxylenol, phenol and thymol). The first approach was based on the optimization of the labelling and LC separation conditions of 1-fluoro-2,4-dinitrobenzene (FDNB), well-known pre-column UV derivatising reagent for amine [9–13]. The compound was also used for phenol analysis by gas chromatography (GC) [13], and by kinetic potentiometric [14] and spectrophotometric [15] procedures, but applications to phenolic drug analysis in reversed phase high-performance liquid chromatography (RP-HPLC) are not reported. In order to develop simple and sensible methods, on the basis of encouraging results of recent research on the post-column photochemical derivatization [6,16,17], the second approach involved the use of a photochemical reactor in combination with the fluorescence or UV-photodiode array detector (DAD). When the photoreactor was arranged on-line between the RP-HPLC column exit and the detector, the column effluent was subjected to UV irradiation (254 nm) and many phenols were converted to photoproducts having modified spectral properties. Thus the UV spectra obtained with the photoreactor 'on' and 'off' for each analyte, provided additional, selective informations useful in confirming the peak identity. Moreover, favourable alterations in the spectral properties were found to improve the analytical detectability of chlorophenols. The developed LC methods were successfully applied to the analysis of phenols in commercial dosage forms.

2. Experimental

2.1. Chemicals and solutions

FDNB, 2-chlorophenol, 2,4-dichlorophenol, 2,6-dichlorophenol, 2,4,5-trichlorophenol, 5-methyl-2-(1-methylethyl)phenol (thymol) and 4-chloro-3,5-dimethylphenol (chloroxylenol) were obtained from Sigma (St. Louis, MO). 3-Chlorophenol, 4-chloro-3-methylphenol (chlorocresol), phenol and hexadecyltrimethylammonium bromide (CTAB) were purchased from Fluka AG (Buchs, Switzerland). 4-Acetylbiphenyl (Internal Standard, I.S.) was from Janssen (Beerse, Belgium). Acetonitrile, methanol, tetrahydrofuran and triethylamine (TEA) for chromatography (RP-HPLC grade) were from Romil (Delchimica Scientific Glassware, Naples, Italy) and double distilled water was used.

Solutions of the reagent FDNB (8 mg ml^{-1}) were prepared in acetone and were found to be stable for 2–3 weeks at 4°C. The standard solutions of phenols used for pre-column derivatization were prepared in water–methanol (98:2, v/v) and the standard solutions used for the direct analysis were prepared in mobile phase (concentration under calibration graphs). Solutions of the IS, 4-acetylbiphenyl (4.70, 2.60, 7.6 and 1.25 mg ml^{-1} for chlorocresol, chloroxylenol, phenol and thymol, respectively) were prepared in acetonitrile. A 0.05 M TEA phosphate buffer (pH 3.0) was prepared by adding orthophosphoric acid to an aqueous TEA solution up to the desired pH and 3 mM CTAB solution in 0.03 M borate buffer, dissolving CTAB in 0.1 M borate buffer (pH. 9.0) obtained according to the standard method [18].

2.2. Equipment

The liquid chromatograph used for direct analysis comprised of a Jasco Model LG-980-02S ternary gradient unit, a Jasco PU-1580 pump and a Jasco FP-920 fluorescence detector connected to a personal computer AcerView 34TL. The integration program Borwin was used. A second liquid chromatograph consisted of a Varian 2010 pump and a Jasco MD-910 diode array detector connected to a personal computer AcerView 54eL. The integration program Borwin-PDA was used. For both chromatographic systems the solvents were degassed on-line with a degasser model Gastorr 153 SAS Corporation (Tokyo, Japan). The liquid chromatograph, used for analysis after chemical derivatization, consisted of a Varian Model 5020 chromatograph and a photometric diode array detector (HP 1040A) connected to an HP 79994A workstation. For any chromatographic system manual injections were carried out using a Rheodyne model 7125 or 7725i injector with 20 μl sample loop. A Beam Boost Model C6808 photoreactor (ICT, Frankfurt, Germany) was arranged on-line between the analytical column and the detectors (UV-DAD or fluorescence). The eluate was irradiated on-line in capillary PTFE tubing (20 m × 0.3 mm i.d.) in a crocheted geometry by an 8 W low-pressure

mercury lamp with the main spectral emission at 254 nm. IR spectra were recorded in a nujol mull on a Perkin-Elmer model 298 apparatus. UV spectra were recorded on a Jasco Uvidec 610 double beam spectrophotometer. ^{1}HNMR spectra were recorded on a Varian Gemini spectrometer at 300 MHz in $CDCL_3$.

2.3. Pre-column derivatization

A 0.5 ml aliquot of the phenol solution was treated with 0.25 ml of 3 mM CTAB solution in 0.03 M borate buffer (pH 9.0) and 50 μl of the reagent (FDNB) solution (8 mg ml^{-1}). The reaction was carried out at 40°C for 20 min under magnetic stirring in a micro reaction vessel (3.0 ml). Then, 1 ml of IS solution in acetonitrile was added; the reaction mixture was stirred for 30 s and 20 μl aliquots of the resulting clear solution were injected into the chromatograph.

When the reaction was performed on preparative scale, a previous method [8] was used and the new ethers were characterised as follows.

2.3.1. 1-(4-Chloro-3-methylphenoxy)-2,4-dinitrobenzene

P.f. 110°C. IR (cm^{-1}): 1620, 1540 (NO_2), 1290 (Ar–O–Ar), 1160, 1060, 885, 850, 820. UV (ethanol) λ_{max} = 289 nm (ε = 8900). ^{1}HNMR ($CDCl_3$): δ (ppm) 2.422 (s, 3H, CH_3), 6.944 (dd, 1H, H–Ar), 7.087–7.041 (m, 2H, H–Ar), 7.452 (d, 1H, H–Ar), 8.347 (dd, 1H, H–Ar), 8.854 (d, 1H, H–Ar).

2.3.2. 1-(4-Chloro-3,5-dimethylphenoxy)-2,4-dinitrobenzene

P.f. 151°C. IR (cm^{-1}): 1600, 1520 (NO_2), 1260 (Ar–O–Ar), 1150, 1020, 870, 820. UV (ethanol) λ_{max} = 290 nm (ε = 9900).^{1}HNMR ($CDCl_3$): δ (ppm) 2.416 (s, 6H, $2CH_3$), 6.898 (s, 2H, H–Ar), 7.063 (dd, 1H, H–Ar), 8.333 (dd, 1H, H–Ar), 8.840 (d, 1H, H–Ar).

2.3.3. 1-(2-Isopropyl-5-methylphenoxy)-2,4-dinitrobenzene

P.f. 71°C. IR (cm^{-1}): 1610, 1540 (NO_2), 1260 (Ar–O–Ar), 1140, 1090, 830, 810. UV (ethanol) λ_{max} = 289 nm (ε = 10670). ^{1}HNMR ($CDCl_3$): δ (ppm) 1.179 (d, 6H, CH_3-iPr), 2.330 (s, 3H, CH_3), 3.009 (m, 1H, CH-iPr), 6.802 (s, 1H, H–Ar), 6.918 (d, 1H, H–Ar), 7.119 (d, 1H, H–Ar), 7.309 (d, 1H, H–Ar), 8.286 (dd, 1H, H–Ar), 8.851 (d, 1H, H–Ar).

2.4. Chromatographic conditions

After chemical derivatization, the LC separations were performed at ambient temperature on a Tracer Spherisorb 5ODS$_2$ (250 mm × 4.6 mm i.d.) column. For routine analyses, a mobile phase consisting of acetonitrile–water (73:27, v/v) at a flow rate of 0.8 ml min^{-1} was used. The LC separations with and without photochemical derivatization were carried out at ambient temperature using a Phenomenex Luna Phenyl-Hexyl (250 mm × 4.6 mm i.d.) column with a mobile phase consisting of 0.05 M TEA phosphate buffer (pH 3.0)–acetonitrile (56:44, v/v) and 0.05 M TEA phosphate buffer (pH 3.0)–acetonitrile–tetrahydrofuran (56:40:4, v/v/v) at a flow rate of 1 ml min^{-1}.

2.5. Calibration graphs

Standard solutions of chlorocresol (3.16–94.7 μg ml^{-1}), chloroxylenol (2.44–97.8 μg ml^{-1}), phenol (3.08–123.3 μg ml^{-1}) and thymol (1.86–74.6 μg ml^{-1}) used for the pre-column derivatization reaction were prepared in a mixture of water–methanol (98:2, v/v). A 0.5 ml volume of the phenol standard solution was subjected to the described derivatization procedure. Triplicate injections for each standard solution were made and the peak-area ratio of the analyte to IS was plotted against the corresponding phenol concentration to obtain the calibration graphs. Standard solutions of phenol (4.53–90.6 ng ml^{-1}), chlorocresol (0.105–5.24 μg ml^{-1}), chloroxylenol (0.844–16.8 μg ml^{-1}) and thymol (0.06–1.80 μg ml^{-1}) used for the analysis with fluorescence detection were prepared in a mixture of 0.05 M TEA phosphate buffer (pH 3)–acetonitrile (56:44, v/v). Triplicate injections for each standard solution were made and the peak-area was plotted against the corresponding phenol concentration to obtain the calibration graphs.

2.6. Analysis of pharmaceutical formulations

The sample preparations were carried out following different procedures as required by the analysis method.

2.6.1. UV-DAD analysis

2.6.1.1. Chlorocresol (ointment) and chloroxylenol (cream). A sample of the commercial pharmaceutical dosage forms equivalent to about 0.80 mg of chlorocresol or 0.40 mg of chloroxylenol was treated separately with 50 ml of methanol–water (25:75, v/v) by ultrasonication for 15 min at 50°C, obtaining lactescent solutions.

2.6.1.2. Thymol (vaginal lavage), chloroxylenol (solution) and phenol (mouthwash). An aliquot of the commercial solutions equivalent to about 4.70 mg of thymol, 0.26 mg of chloroxylenol and 0.26 mg of phenol was diluted in methanol–water (70:30, v/v) and then in water (thymol) and in acetonitrile–water (25:75, v/v) (chloroxylenol and phenol) to give the final solutions.

The sample solutions (clear or lactescent) were subjected to the pre-column chemical derivatization with the described FDNB solution and analysed by comparison with an appropriate standard solution of derivatised phenols (chlorocresol: 16 $\mu g\,ml^{-1}$; chloroxylenol: 8 and 13 $\mu g\,ml^{-1}$ for cream and solution, respectively; thymol: 24 $\mu g\,ml^{-1}$; phenol: 13 $\mu g\,ml^{-1}$). UV detection at 292 nm.

2.6.2. Fluorimetric analysis

2.6.2.1. Chlorocresol (cream) and chloroxylenol (cream). An amount of the cream equivalent to about 0.10 mg of chlorocresol and 0.40 mg of chloroxylenol, accurately weighed into a centrifuge tube, was extracted (3×2 and 2×4 ml for chlorocresol and chloroxylenol, respectively) with a mixture consisting of 0.05 M TEA phosphate buffer (pH 3)–methanol (70:30 and 50:50 v/v, for chlorocresol and chloroxylenol, respectively) by ultrasonication for 10 min. After centrifugation for 10 min at 3000 rpm, the supernatants were filtered and combined quantitatively into a volumetric flask and diluted to 20 ml (chlorocresol) and 10 ml (chloroxylenol) with the mixture used for the extraction. Then, 0.5 ml (chlorocresol) and 1 ml (chloroxylenol) aliquot of these solutions were diluted with the mobile phase to obtain the expected concentrations.

2.6.2.2. Thymol (vaginal lavage), chloroxylenol (solution) and phenol (mouthwash). An aliquot of the commercial solution equivalent to about 4.70 mg of thymol, 0.35 mg of chloroxylenol and 0.36 mg of phenol was diluted to 20 ml with mobile phase (thymol) and 10 and 20 ml volume with ethanol for chloroxylenol and phenol, respectively. Then, 1 ml aliquot of these solutions was diluted with 10 and 5 ml of mobile phase (for thymol and chloroxylenol, respectively) and with 10 ml of ethanol–mobile phase (50:50, v/v) (for phenol). Finally, 0.1 ml of the thymol and phenol solutions were diluted with mobile phase to obtain the desired concentrations.

The sample solutions were filtered through a 0.45 μm nylon 25 mm filter and subjected to LC analyses by direct fluorescence detection at $\lambda_{em} = 310$ nm with $\lambda_{ex} = 270$ nm; the phenol content in each sample was determined by comparison with an appropriate standard solution (chlorocresol: 0.25 $\mu g\,ml^{-1}$; chloroxylenol: 8 and 7 $\mu g\,ml^{-1}$ for cream and solution, respectively; phenol: 18 $\mu g\,ml^{-1}$; thymol: 0.24 $\mu g\,ml^{-1}$).

3. Results and discussion

3.1. Pre-column derivatization reaction

The derivatization reaction was carried out according to previous studies [14,15] in an aqueous medium. To achieve optimum conditions, the effects of the temperature, reagent and CTAB micelles on the reaction were investigated. CTAB micelles proved to be a useful device to enhance the reaction rate; when CTAB was omitted, the reaction times were significantly longer. Under the described conditions, the reaction was found to be complete and essentially quantitative after 2 min at room temperature and at a molar ratio of reagent to phenol of about 10 and a further reagent excess did not interfere. As higher temperatures did not give degradation products, the reaction was carried out at 40°C for 20 min to assure the complete reactivity of phenol present in complex matrices. The reaction yield was found to be about 100–102% by comparison with authentic specimens of chlorocresol, chloroxylenol and thymol ethers.

3.2. Chromatography and detection

3.2.1. Pre-column chemical derivatization

Chromatographic separations were carried out under isocratic conditions at ambient temperature on a reversed phase column (RP-18) with UV-DAD (λ = 292 nm). The composition of the mobile phase was evaluated to optimise the resolution of the ethers. A mobile phase consisting of acetonitrile–water (73:27, v/v) at a flow rate of 0.8 ml min^{-1} was found to be suitable. A representative LC separation of the derivatised phenols is reported in Fig. 1. As can be seen, the reagent and solvent did not interfere with the analysis because they were eluted close the solvent front and before the derivatised phenols. Under the chosen conditions, the detection limit (signal-to-noise (*S/N*) ratio = 3) was about of 2–10 pmol for different phenols. The derivatization technique offers both, the opportunity to achieve useful qualitative informations on the analyte identity and to enhance the sensitivity by three to nine times. As example in Fig. 2, the UV spectra of derivatised phenol and chloroxylenol are reported in comparison with those of starting compounds.

3.2.2. Post-column photochemical derivatization

The LC separations were carried out under isocratic conditions at ambient temperature on a reversed phase column (Phenyl-Hexyl) with UV-DAD (λ = 270 nm) and fluorescence (λ_{ex} = 270 nm, λ_{em} = 310 nm) detection. The effects of composition and pH of the mobile phase on the resolution and fluorescence intensity of phenols were investigated. A solvent mixture of 0.05 M TEA phosphate buffer (pH 3.0)–acetonitrile–tetrahydrofuran (56:40:4, v/v/v) at a flow rate of 1 ml min^{-1} allowed to obtain the acceptable resolution for all analytes (Fig. 3). In order to provide an information-rich detection, a UV-DAD and a fluorescence detector were used in combination with the post-column on-line photochemical derivatization. Typical chromatograms obtained with the on-line photoreactor 'on' and 'off' exhibit different profiles with selectively alterated peak heights with UV-DAD detection (Fig. 3). In particular, significant

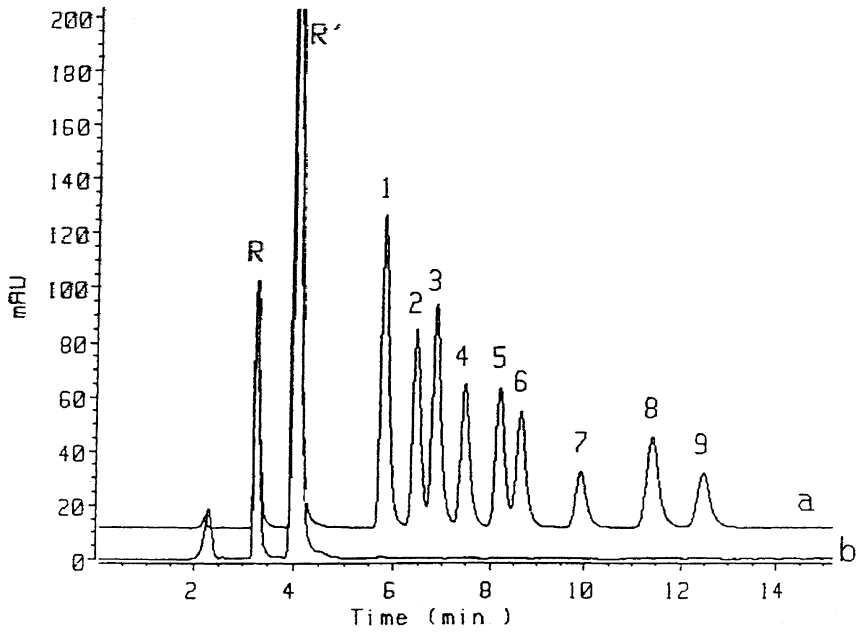

Fig. 1. LC chromatogram at ambient temperature of (a) phenol mixture derivatized with FDNB and (b) reagent (FDNB) under reaction conditions. Peaks: R = acetone; R′ = FDNB; (1) phenol; (2) 2-chlorophenol; (3) 3-chlorophenol; (4) 2,6-dichlorophenol; (5) 2,4-dichlorophenol; (6) chlorocresol; (7) 2,4,5-trichlorophenol; (8) chloroxylenol; (9) thymol. Column: Tracer Spherisorb 5ODS$_2$ (250 mm × 4.6 mm i.d.). Mobile phase: acetonitrile–water (73:27, v/v) at a flow rate of 0.8 ml min^{-1}. UV-DAD detection (λ = 292 nm).

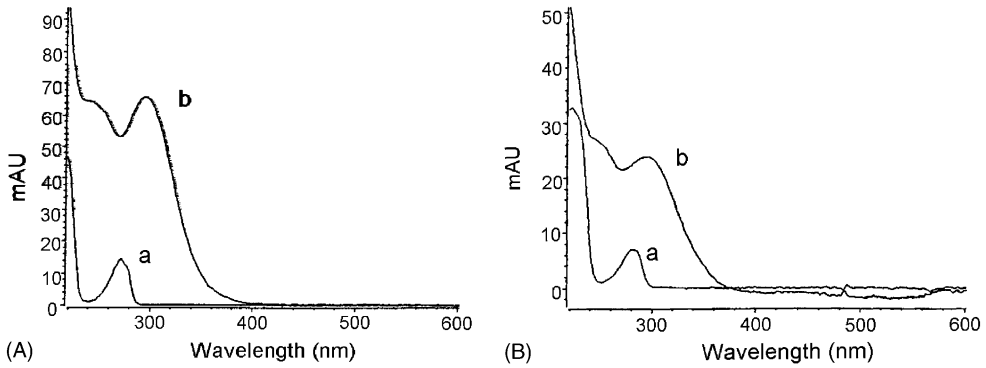

Fig. 2. On-line UV spectra of phenol (A) and chloroxylenol (B) before (a) and after (b) pre-column derivatization with FDNB. Chromatographic conditions and detection are same as in Fig. 1.

increase of the peaks of phenol, chlorocresol, chloroxylenol and 2,4,5-trichlorophenol was observed. In view of positive identification of the chromatographic peaks, additional informations on the analyte structure and photoreactivity, obtained by UV spectra (Fig. 4), are useful to support the chromatographic retention data. These informations, in combination with the retention data, offer an important basis for a practical and reliable quality control of the commercial formulations.

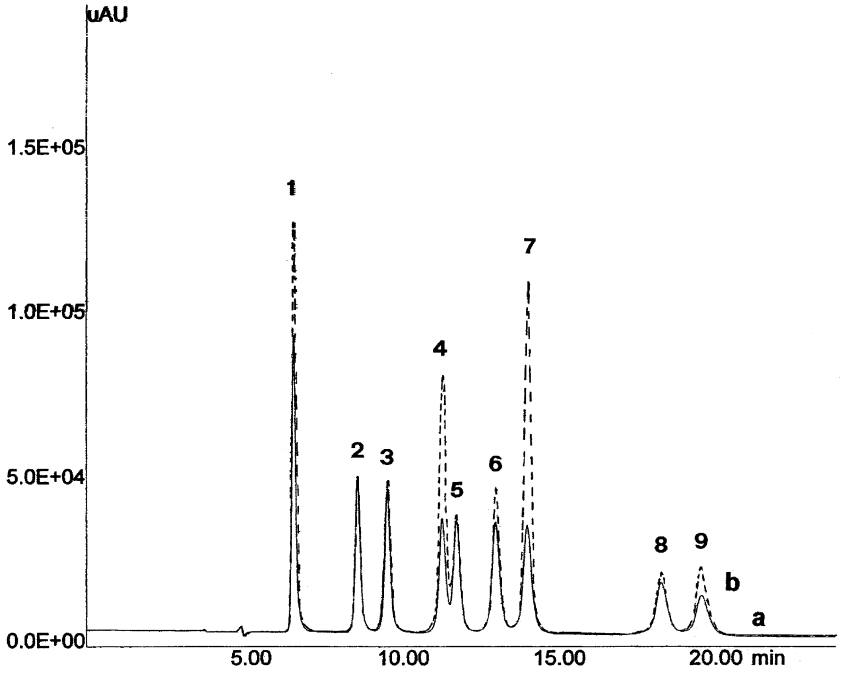

Fig. 3. Representative LC separation of phenols with on-line photoreactor switched (a) 'off' and (b) 'on'. Peaks: (1) phenol; (2) 2-chlorophenol; (3) 3-chlorophenol; (4) chlorocresol; (5) 2,6-dichlorophenol; (6) 2,4-dichlorophenol; (7) chloroxylenol; (8) thymol; (9) 2,4,5-trichlorophenol. Column: Phenomenex Luna Phenyl-Hexyl (250 mm × 4.6 mm i.d.). Mobile phase: 0.05 M TEA phosphate buffer (pH 3.0)–acetonitrile–tetrahydrofuran (56:40:4, v/v) at a flow rate of 1 ml min^{-1}. UV-DAD detection: $\lambda = 270$ nm.

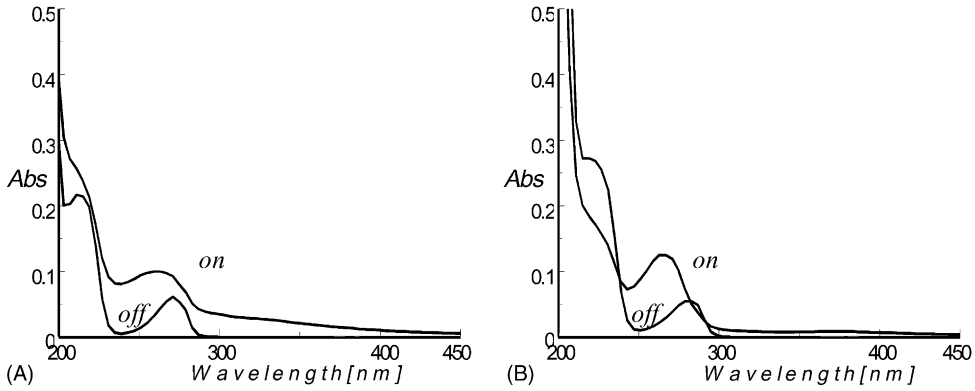

Fig. 4. UV spectra of phenol (A) and chloroxylenol (B) with on-line photoreactor switched 'off' and 'on'. Chromatographic conditions and detection as in Fig. 3.

Moreover, after photochemical reaction with fluorescence detection with a mobile phase consisting of 0.05 M TEA phosphate buffer (pH 3.0)–acetonitrile (56:44, v/v), the chlorocresol and chloroxylenol peaks increase and it is of great interest the fluorescence intensity of 2-chlorophenol, 3-chlorophenol, 2,6-dichlorophenol, 2,4-dichlorophenol and 2,4,5-trichlorophenol, without native fluorescence (Fig. 5

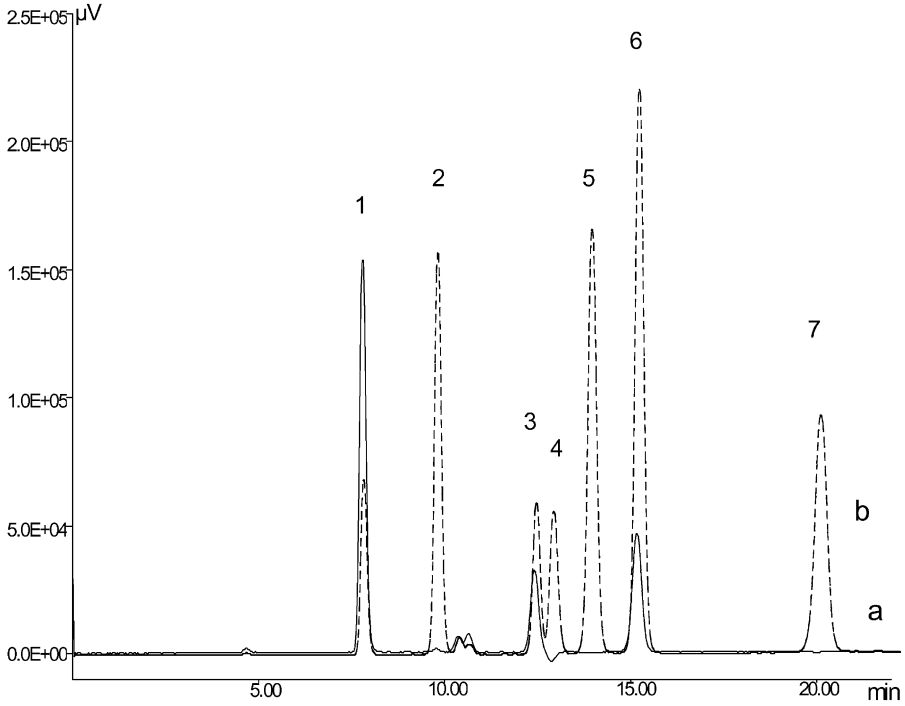

Fig. 5. Representative LC separation of phenols with on-line photoreactor switched (a) 'off' and (b) 'on'. Peaks: (1) phenol; (2) 2-chlorophenol; (3) chlorocresol; (4) 2,6-dichlorophenol; (5) 2,4-dichlorophenol; (6) chloroxylenol; (7) 2,4,5-trichlorophenol. Column: Phenomenex Luna Phenyl-Hexyl (250 mm × 4.6 mm i.d.). Mobile phase: 0.05 M TEA phosphate buffer (pH 3.0)–acetonitrile (56:44, v/v) at a flow rate of 1 ml min^{-1}. Fluorescence detection: $\lambda_{em} = 310$ nm with $\lambda_{ex} = 270$ nm.

Table 1
Detection limit of phenols ($S/N = 3$)

Compound	RP-HPLC-fluorimetric method $\lambda_{ex} = 270$ nm, $\lambda_{em} = 310$ nm (pmol)		RP-HPLC-UV method $\lambda = 270$ nm (pmol)	
	Photoreactor 'off'	Photoreactor 'on'	Photoreactor 'off'	Photoreactor 'on'
Phenol	0.030	0.067	27	14
Thymol	0.047	0.11	33	25
Chlorocresol	0.35	0.21	38	19
Chloroxylenol	4.7	1.6	58	12
3-Chlorophenol	–[a]	0.52	55	55
2-Chlorophenol	–	1.6	16	16
2,6-Dichlorophenol	–	90	25	25
2,4-Dichlorophenol	–	88	48	32
2,4,5-Trichlorophenol	–	33	110	55

[a] – : Not detectable.

and Table 1). Unfortunately, under these chromatographic conditions, thymol coelutes with 2,4,5-trichlorophenol and 3-chlorophenol with 2-chlorophenol; in the presence of 4% tetrahydrofuran in the mobile phase (Fig. 3), a good separation was obtained, but a minor favourable increasing of fluorescence intensity for the different chlorophenols was recorded. Preliminary investigations have been performed on the photo-induced modification of these chlorophenols. For this study, the photoreactor was arranged between the pump and the analytical column to observe the chromatographic behaviour of the chlorophenols after photochemical derivatization. In particular, the partial conversion of 2-chlorophenol to phenol suggests a probable chloro elimination from the aromatic structure under irradiation at 254 nm, resulting in the enhancement of fluorescence intensity. The halogen removal, depending on the chloro substituent position on the aromatic ring and the developing of other potential degradation products under irradiation can account for the fluorescence increase of some chlorophenols. Further studies are in progress for the confirmation. The detection limit (signal-to-noise (*S/N*) ratio = 3) with photoreactor 'off' and 'on' is reported in Table 1. As can be seen, the method with fluorimetric detection is more sensitive than the procedure with UV detection for phenol, thymol, chlorocresol and chloroxylenol both before and after photochemical derivatization. Moreover, using photoreactor 'on', 2-chlorophenol and 3-chlorophenol are more detectable with fluorescence detection that with UV detection and the dichlorophenols and the trichlorophenol exhibit practically comparable detectivity. However, the fluorescence intensity increases significantly for all chlorophenols (five to ten times) after long irradiation times at low flow rates; as example, the chlorocresol behaviour is reported in Fig. 6.

3.3. Analysis of pharmaceutical formulations

Routine analyses of the phenols in their commercial dosage forms containing a small percentage of chlorocresol (0.1%, w/w), chloroxylenol (0.3 or 0.4%, w/w), phenol (0.73%, w/w) and thymol (0.47% w/v) were carried out by pre-column derivatization with FDNB and UV-DAD (method A) and by fluorimetric analysis with photoreactor 'off' (method B). Under the described chromatographic conditions, linear relationships were found for each phenol (Table 2). As can be seen, the within-run precision (repeatability) of the derivatization and LC separation (method A) and LC assay (method B), expressed as R.S.D. from replicate ($n = 8$) analyses of the same standard solution was satisfactory for each compound. The subsequent analysis of the commercial formulations did not involve a particular preliminary sample preparation, but a mild heating at 50°C for 15 min (method A) and a simple centrifugation (method B) are sufficient in order to remove interfering components of complex matrices as cream or ointment. The phenol content in each commercial dosage form agreed with the claimed content with good within-run precision (repeatability). The recovery was between 94.6 and 101.5% for each compounds (R.S.D. between 0.8

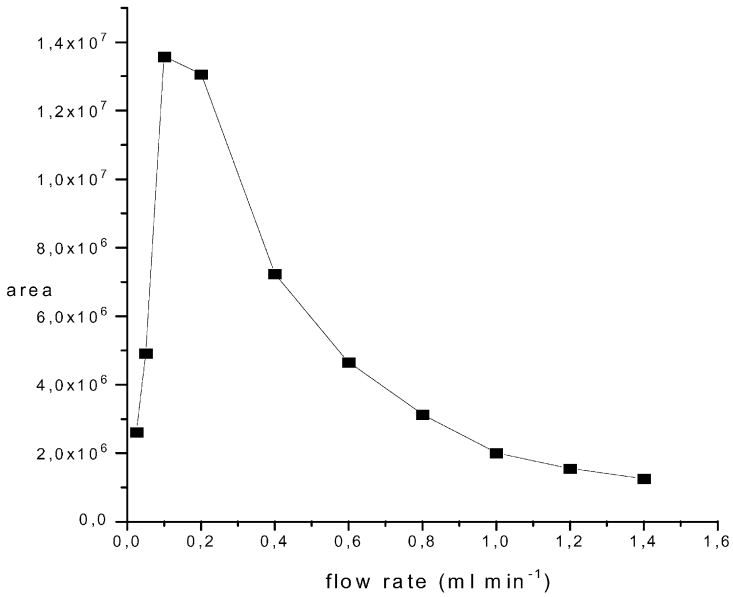

Fig. 6. Effect of flow rates on the fluorescence intensity of chlorocresol with on-line photoreactor switched 'on'. Mobile phase and detection as described in Fig. 5.

and 2.5%). The other ingredients of the formulations did not interfere with the analysis. The accuracy of the whole method (pre-column derivatization and LC or direct LC) was verified by analysing samples fortified with 30 and 50% of the claimed phenol content; the recoveries were 97.0–102%. The results obtained with the method A were in agreement with those of the method B. An example of chromatogram obtained from a commercial sample is illustrated in Fig. 7.

Table 2
Data for calibration graphs ($n = 6$) and repeatability of phenols

Compounds	Method[a]	Slope[b]	y-Intercept[b]	Correlation coefficient	Concentration range ($\mu g\,ml^{-1}$)	R.S.D. (%)[c]
Chlorocresol	A	0.06490	−0.003872	0.9999	3.16–94.7	1.0
	B	614968	106528	0.9999	0.105–5.24	0.42
Chloroxylenol	A	0.08344	0.01696	0.9999	2.44–97.8	0.51
	B	178429	471304	0.9997	0.844–16.8	0.13
Phenol	A	0.08682	0.02347	0.9999	3.08–123.3	0.58
	B	27472	510154	0.9998	4.53 to 90.6×10^{-3}	0.36
Thymol	A	0.2725	−0.09470	0.9998	1.86–74.6	0.63
	B	14156645	22279	0.9999	0.06–1.80	0.39

[a] A, LC analysis by pre-column derivatization with FDNB and UV-DAD detection; B, LC analysis with fluorimetric detection.

[b] According to $y = ax + b$, where x is the concentration, y the ratio of analyte peak-area to IS peak-area (method A) and y the peak-area of phenol expressed in 10^5 to 10^8 μV (method B).

[c] Within-run precision (repeatability) of the derivatization and LC separation for method A and LC assay for method B.

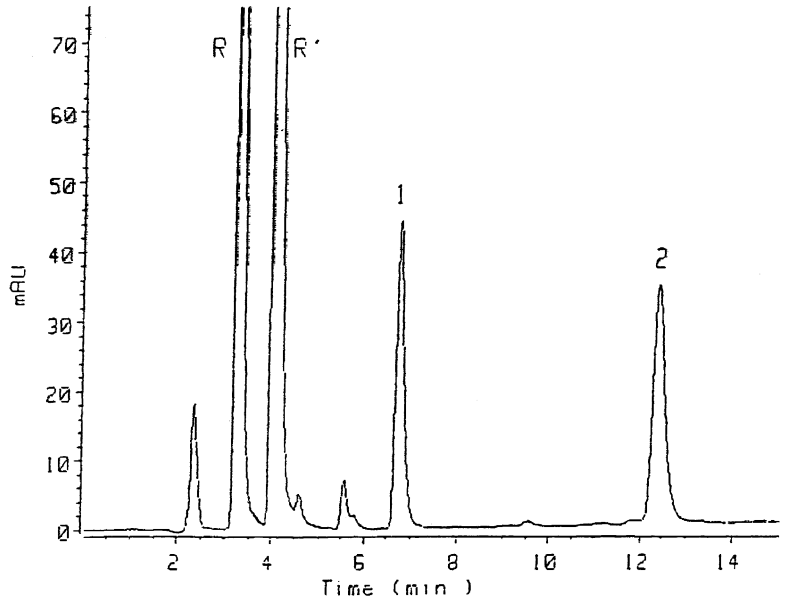

Fig. 7. LC chromatogram at ambient temperature of a sample of vaginal lavage after chemical derivatization with FDNB. Peaks: R = acetone; R′ = reagent; (1) acethylbiphenyl (IS); (2) thymol. Chromatographic conditions and detection are same as in Fig. 1.

4. Conclusions

The proposed RP-HPLC methods were shown to be useful for the quality control of pharmaceutical formulations containing very low levels of phenols. FDNB has been found to be an useful reagent for pre-column chromatographic analysis of phenol drugs. Both pre-column chemical derivatization and on-line post-column photochemical derivatization are reliable means of an unambiguous identification of these compounds. The photochemical reactor in combination with a fluorimetric detector can enhance not only the sensitivity of the method, but offer the opportunity to identify chlorophenols without native fluorescence. The described procedures are simple and allow the analysis of phenols by mild conditions in commercial dosage forms of complex composition without laborious extraction methodologies.

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