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HPLC-fluorescence determination of chlorocresol and chloroxylenol in pharmaceuticals

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Abstract

The use of 2-chloro-6,7-dimethoxy-3-quinolinecarboxaldehyde as a fluorogenic labelling reagent in pre-column derivatization for the HPLC separation of chlorophenols has been investigated. The compound reacts (50 min at 110°C) with 2- and 4-chlorophenols to give fluorescent ethers that can be separated by reversed-phase HPLC and detected at $\lambda_{exc} = 360$ nm, $\lambda_{em} = 500$ nm. The experimental conditions for derivatization and chromatographic separation are discussed. Applications for the determination of chlorocresol (4-chloro-3-cresol) and chloroxylenol (4-chloro-3,5-xylenol) in pharmaceutical formulations (creams, ointments) are described. © 1997 Elsevier Science B.V.

Keywords: Chlorophenols; Reversed phase liquid chromatography; Fluorogenic pre-column derivatization; 2-Chloro-6,7-dimethoxy-3-quinolinecarboxaldehyde

1. Introduction

Many pharmaceutical formulations (creams, ointments) used for the treatment of various skin infections contain low concentrations (0.1–0.4% w/w) of chlorocresol (4-chloro-3-cresol) or chloroxylenol (4-chloro-3,5-xylenol) as preservatives; control of their level is desiderable to assure the quality of the products.

For the analysis of chlorocresol in pharmaceuticals, methods based on HPLC [1], enzyme sensor [2] and flow injection analysis (FIA) [3] have been proposed. Liquid chromatographic (HPLC and TLC) methods have also been reported for the determination of various preservatives in cosmetics [4–6] and aluminium-backed paper [7]. A direct HPLC method (UV detection) [1] proved to be suitable for the analysis of pharmaceutical creams; however, a selective and practical pre-chromatographic derivatization is of interest, because the HPLC method performance can be improved and a wider application yield can be obtained.

Fluorogenic derivatization prior to HPLC separation constitutes a widespread, effective technique to improve analysis sensitivity and selectivity. A great variety of reagents has been developed for the derivatization of amines, thiols and carboxylic acids, but few probes have been proposed for the determination of hydroxy compounds, whether alcohols or phenols [8,9].

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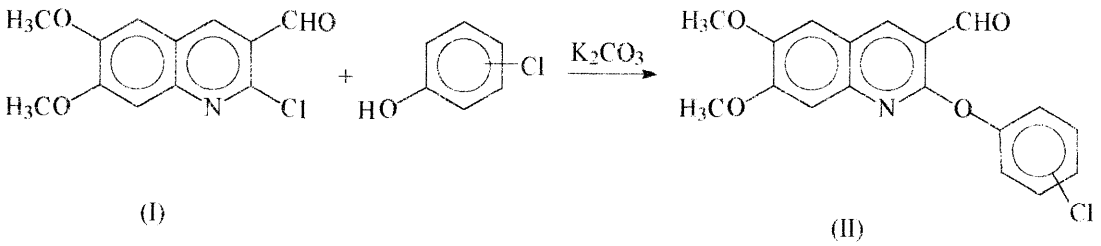

(I) (II)

Fig. 1. Derivatization reaction of chlorophenols with 2-chloro-6,7-dimethoxy-3-quinolinecarboxaldehyde (I).

In the present study, 2-chloro-6,7-dimethoxy-3-quinolinecarboxaldehyde (I) is proposed as a useful fluorogenic reagent for precolumn derivatization in the HPLC analysis of chlorophenols (Fig. 1). This paper deals with the application of reagent (I) to the determination of chlorocresol and chloroxylenol in pharmaceutical dosage forms of complex composition. The method allows a selective derivatization of these preservatives in the diluted matrix, without the need of preliminary liquid-liquid or solid-phase extraction procedures. For its advantages of selectivity and sensitivity (fluorimetric detection) the method can be considered of real interest for reliable and practical quality control of pharmaceutical formulations.

2. Experimental

2.1. Materials

2-Chlorophenol, 4-chlorophenol, chloroxylenol (4-chloro-3,5-xylenol; 4-chloro-3,5-dimethylphenol), 2,4-dichlorophenol, were from Sigma (St. Louis, MO). Chlorocresol (4-chloro-*m*-cresol; 4-chloro-3-methylphenol) was from Fluka AG (Buchs, Switzerland) and all the other chemicals were from Carlo Erba (Italy). The reagent 2-chloro-6,7-dimethoxy-3-quinolinecarboxaldehyde (Cl-DQCA) was prepared and purified as previously described [10]. Organic solvents for chromatography were of HPLC grade (Mallinkrodt, USA) and deionized double distilled water was used.

2.2. Apparatus

The liquid chromatograph comprised a Varian 2010 pump and a Varian 2070 fluorescence spectrophotometer, operating at an emission wavelength of 500 nm with an excitation wavelength of 360 nm, connected to a personal computer IBM XT-PC. The integration program 'JCL6000 chromatography data system' was used. Manual injections were carried out using a Rheodine model 7125 injector with a 50 μl sample loop. The solvents were degassed on line with a degasser model ERC-3312 from Erma, (Tokyo, Japan).

IR spectra were recorded in a nujol mull on a Perkin-Elmer model 298 apparatus. UV spectra ($c = 0.45 \times 10^{-4}$ M) were recorded on a Jasco Uvidec 610 double beam spectrophotometer.

^{1}HNMR spectra were recorded on a Varian Gemini spectrometer at 300 MHz in $CDCl_3$.

A Reacti-Therm heating/stirring (Pierce, USA), was used for the chemical derivatization.

Sonarex Super RK 102 (35 KHz) Bandelin (Berlin, Germany) equipment with termostatically controlled heating (30–80°C) was used for ultrasonication.

2.3. Synthesis of 2-phenoxyquinolines (II)

General procedure: the phenol (0.39 mmol) in about 5 ml *N*,*N*-dimethylformamide was treated with 0.42 mmol of the reagent 2-chloro-6,7-dimethoxy-3-quinolinecarboxaldehyde (Cl-DQCA), in the presence of 100 mg potassium carbonate for 1.5 h at reflux. After cooling, the

reaction mixture was diluted with 30 ml water and neutralized with 2 N acetic acid (60:40, v/v). The precipitate was collected by filtration and purified by cristallization from acetone and water to give a white compound which was found to be homogeneous by TLC on silica gel using ethylacetate–petroleum ether (4:6, v/v). UV detection was at 254 and 366 nm.

2.3.1. 2-(2-Chlorophenoxy)-6,7-dimethoxy-3-quinolinecarboxaldehyde

M.p. 202–205°C; calculated for $C_{18}H_{14}ClNO_4$, C 62.89, H 4.10, N 4.07; found C 62.60, H 4.35, N 4.20. IR (cm^{-1}): 1675, 1590, 1250, 1220, 1120, 1000, 855, 845. ^{1}HNMR ($CDCl_3$): δ 4.00 (2s, 6 H, 2 OCH_3), 7.05–7.40 (m, 6H, quinoline H-5, H-8 and phenoxy H-3, H-4, H-5, H-6), 8.62 (s, 1 H, quinoline H-4), 10.68 (s, 1 H, CHO). UV (ethanol) $\lambda_{max} = 321$ nm ($\varepsilon = 1.088 \times 10^4$), 360 nm ($\varepsilon = 0.550 \times 10^4$).

2.3.2. 2-(4-Chlorophenoxy)-6,7-dimethoxy-3-quinolinecarboxaldehyde

M.p. 195–196°C; calculated for $C_{18}H_{14}ClNO_4$, C 62.89, H 4.10, N 4.07; found C 62.64, H 4.04, N 4.10. IR (cm^{-1}): 1675, 1600, 1250, 1215, 1160, 1120, 845. ^{1}HNMR ($CDCl_3$): δ 4.00 (2s, 6H, 2 OCH_3), 7.11–7.50 (m, 6H, quinoline H-5, H-8 and phenoxy H-2, H-3, H-5, H-6), 8.62 (s, 1H, quinoline H-4), 10.60 (s, 1H, CHO). UV (ethanol) $\lambda_{max} = 321$ nm ($\varepsilon = 1.142 \times 10^4$), 360 nm ($\varepsilon = 0.655 \times 10^4$).

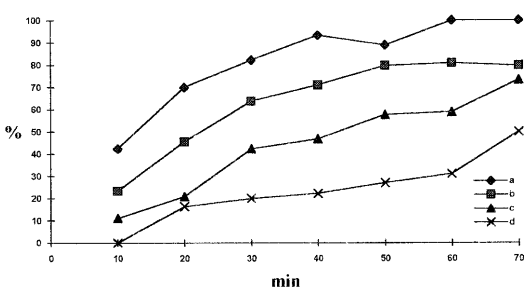

Fig. 2. Influence of the temperature on the derivatization reaction of chlorocresol with the reagent (I): (a) 120°C, (b) 110°C, (c) 100°C using 0.03 ml 60 mM K_2CO_3 each; and (d) 120°C using 0.10 ml 20 mM NaOH. %, Percent yield of the reaction.

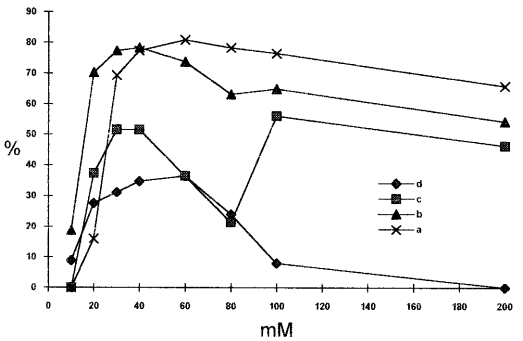

Fig. 3. Effect of the concentration and volume of K_2CO_3 and NaOH solutions on the derivatization reaction (50 min 110°C) of chlorocresol with the reagent (I): (a) 0.03 ml, (b) 0.05 ml, (c) 0.10 ml of K_2CO_3 solution; and (d) 0.10 ml of NaOH solution. %, Percent yield of the reaction.

2.3.3. 2-(4-Chloro-3-methylphenoxy)-6,7-dimethoxy-3-quinolinecarboxaldehyde

M.p. 182–184°C; calculated for $C_{19}H_{16}ClNO_4$, C 63.78, H 4.51, N 3.91; found C 63.50, H 4.62, N 4.10. IR (cm^{-1}): 1675, 1590, 1260, 1250, 1120, 1000, 850, 835, 825. ^{1}HNMR ($CDCl_3$): δ 2.42 (s, 3H, CH_3), 4.00 (2s, 6H, 2 OCH_3), 7.05–7.45 (m, 5H, quinoline H-5, H-8 and phenoxy H-2, H-5, H-6), 8.58 (s, 1 H, quinoline H-4), 10.57 (s, 1 H, CHO). UV(ethanol) $\lambda_{max} = 322$ nm ($\varepsilon = 1.266 \times 10^4$), 360 nm ($\varepsilon = 0.599 \times 10^4$).

2.3.4. 2-(4-Chloro-3,5-dimethylphenoxy)-6,7-dimethoxy-3-quinolinecarboxaldehyde

M.p. 217–220°C; calculated for $C_{20}H_{18}ClNO_4$ C 64.61, H 4.87, N 3.77; found C 64.74, H 4.96, N 3.90. IR (cm^{-1}): 1675, 1595, 1250, 1215, 1125, 1020, 1005, 870, 850, 835. ^{1}HNMR ($CDCl_3$): δ 2.40 (s, 6H, 2 CH_3), 4.00 (2s, 6H, 2 OCH_3), 6.98 (s, 2H, phenoxy H-2, H-6), 7.10 (d, 2H, quinoline H-5, H-8), 8.55 (s, 1H, quinoline H-4), 10.55 (s, 1H, CHO). UV(ethanol) $\lambda_{max} =$ 322 nm ($\varepsilon = 1.240 \times 10^4$), 360 nm ($\varepsilon = 0.537 \times 10^4$).

2.4. Solutions

Solutions of the reagent Cl-DQCA (2 mg ml^{-1} for chlorocresol and 2.5 mg ml for chloroxylenol) were prepared in dimethylsulphoxide (DMSO) and were found to be stable for 1–2 weeks at ambient temperature. Stock solutions of chlorophenols were prepared in DMSO (concentration under calibration graphs). Solutions of the internal standards (IS), 2-(4-chlorophenoxy)-6,7-dimethoxy-3-quinolinecarboxaldehyde (25.5 μg ml^{-1}) and 2-(4-chloro-3-methylphenoxy)-6,7-dimethoxy-3-quinolinecarboxaldehyde (28.0 μg ml^{-1}) were prepared in the mobile phase.

2.5. Derivatization procedure

A 0.2 ml aliquot of the chlorophenol solution was treated with 0.03 ml of 60 mM potassium carbonate aqueous solution with ultrasonication for 10 min at ambient temperature; then 0.4 ml of the reagent solution were added and the reaction was carried out at 110°C for 50 min under magnetic stirring in a micro reaction vessel (3.0 ml). Then 0.06 ml of 2 N acetic acid and 0.8 ml of the appropriate IS solution in the mobile phase were added; the reaction mixture was sonicated for 1 min and 50 μl aliquots of the resulting clear solution were injected into the chromatograph.

2.6. Chromatographic conditions

The HPLC separations were performed at 35°C on a Hypersil 5ODS (250 mm × 4.6 mm i.d.) stainless steel column under isocratic conditions. For routine analyses of chlorocresol, a mobile phase consisting of acetonitrile–triethylammonium phosphate buffer (pH 3.0; 0.05 M) (52:48, v/v) at a flow rate of 1.0 ml min^{-1} was used. The analysis of chloroxylenol was carried out using a mobile phase of mixtures A–B (52:48, v/v), where A is acetonitrile–tetrahydrofuran (95:5, v/v) and B is triethylammonium phosphate buffer (pH 3.0; 0.05 M) at a flow rate of 1.3 ml min^{-1}. The separation of standard mixtures of chlorophenols were performed under isocratic conditions using a mobile phase of acetonitrile–triethylammonium phosphate buffer (pH 3.0; 0.05 M) (48:52, v/v) at a flow rate of 1.3 ml min^{-1}.

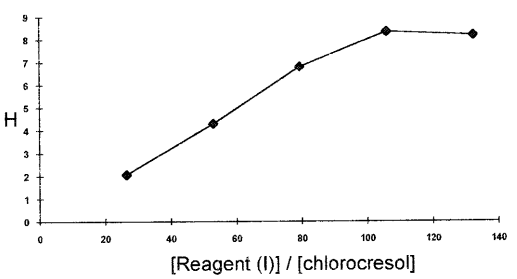

Fig. 4. Effect of the reagent (I)/chlorocresol molar ratio on the derivatization reaction (50 min 110°C with 0.03 ml 60 mM K_2CO_3). H, heigth of the analyte peak.

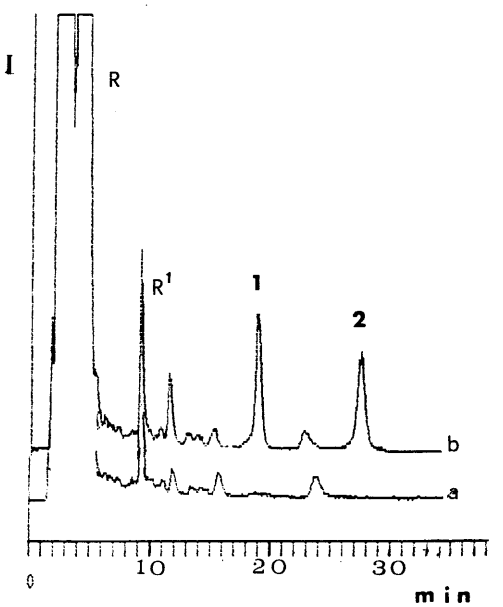

Fig. 5. HPLC chromatogram at 35°C of: (a) reagent (I) under reaction conditions; (b) chloroxylenol and internal standard chlorocresol derivatized with the reagent (I). Peaks: 1, chlorocresol; 2, chloroxylenol; R, R^1 = reagent. Column: Hypersil 5ODS (250 mm × 4.6 mm i.d.). Mobile phase: mixture A–B (52:48, v/v), where A is acetonitrile–tetrahydrofuran (95:5, v/v) and B is triethylammonium phosphate buffer (pH 3.0; 0.05 M) at a flow rate of 1.3 ml min^{-1}. Fluorescence detection: $\lambda_{exc} = 360$ nm; $\lambda_{em} = 500$ nm. I, fluorescence intensity.

2.7. Calibration graphs

Standard solutions of chlorocresol (26.10–130.50 nmol ml^{-1}) and chloroxylenol (48.40–242.00 nmol ml^{-1}) were prepared in DMSO. A 0.2 ml volume of the chlorophenol standard solution was subjected to the described derivatization procedure. Triplicate injection for each standard solution were made and the peak-height ratio of the analite to IS was plotted against the corresponding chlorophenol concentration to obtain the calibration graph.

2.8. Analysis of pharmaceutical formulations

An amount of the commercial pharmaceutical dosage forms (ointment and/or cream) equivalent to about 0.15 mg chlorocresol or 0.23 mg chloroxylenol was treated with 10 ml DMSO by ultrasonication at 40°C for 10 min. After decanting, a 0.2 ml aliquot of the resulting clear solution was subjected to the derivatization reaction with the appropriate Cl-DQCA solution.

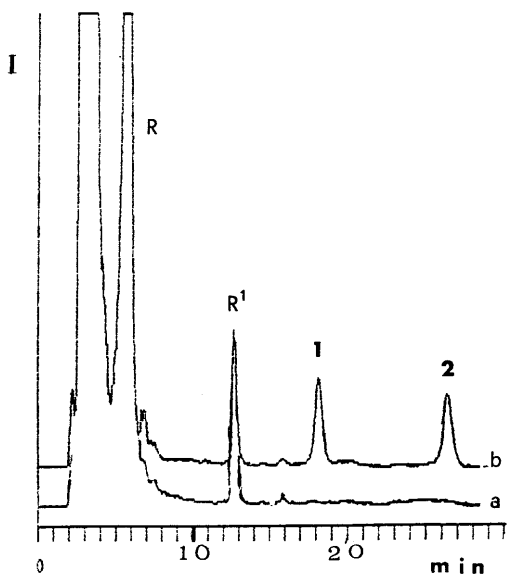

Fig. 6. HPLC chromatogram at 35°C of: (a) reagent (I) under reaction conditions; (b) chlorocresol and IS 4-chlorophenol derivatized with the reagent (I). Peaks: 1, 4-chlorophenol; 2, chlorocresol; R, R^1 = reagent. Column: as in Fig. 5. Mobile phase: acetonitrile–triethylammonium phosphate buffer (pH 3.0; 0.05 M) (52:48, v/v) at a flow rate of 1.0 ml min^{-1}. Detection as in Fig. 5.

3. Results and discussion

The potential of 2-chloro-6,7-dimethoxy-3-quinolinecarboxaldehyde as a fluorogenic derivatization reagent was studied with chlorocresol and chloroxylenol as representative bioactive chlorophenols whose detectability requires to be enhanced. The reagent bears a 3-carboxaldehyde substituent which enhance the reactivity of the C-2 position towards nucleophiles and maintains a favourable effect on the derivative fluorescence.

The reaction with chlorocresol, chloroxylenol, 2-chlorophenol and 4-chlorophenol was first carried out on a preparative scale and the analytical data for the ethers obtained were consistent with the general structure (II). The ethers were then used to verify the yield of the derivatization reaction for analytical applications or as the IS.

3.1. Derivatization reaction

The derivatization reaction (Fig. 1) was studied with chlorocresol, using DMSO as a solvent suitable for nucleophile substitution. To achieve optimum conditions the effects of the temperature and the reagent and potassium carbonate concentration on the reaction were investigated. The course of the reaction at different temperatures under magnetic stirring for chlorocresol is illustrated in Fig. 2. Reaction at 110°C for 50 min, using 0.03 ml 60 mM potassium carbonate using a 110 molar ratio of reagent to chlorophenol, proved to be the best condition; the reaction yield was found to be about 80% by comparison with an authentic specimen of chlorocresol ether. The derivatization of chlorocresol was complete and essentially quantitative in 60 min at 120°C, but in these conditions degradation products increased. With 0.03 ml 60 mM potassium carbonate (Fig. 3) the reaction was complete (not quantitative) and reproducible; higher volumes and concentrations did not offer significant advantages and more degradation products were obtained. Using sodium hydroxide instead of potassium carbonate

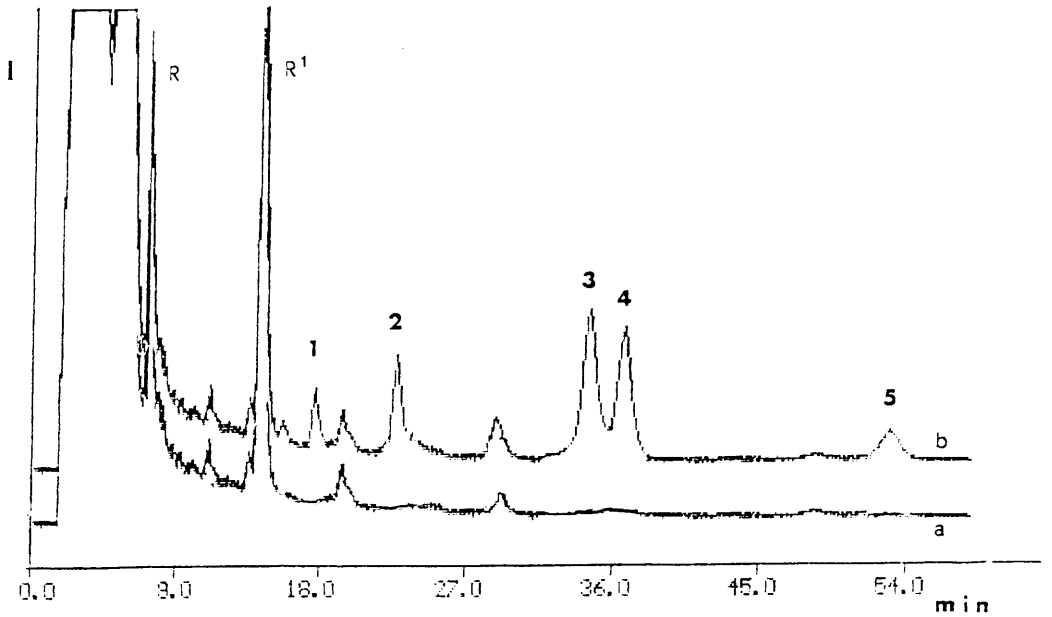

Fig. 7. Representative HPLC separation at 35°C of: (a) reagent (I) under reaction conditions; (b) chlorophenols derivatized with the reagent (I). Peaks: 1, 2-chlorophenol; 2, 4-chlorophenol; 3, chlorocresol; 4, 2,4-dichlorophenol; 5, chloroxylenol; R, R^1 = reagent. Column: as in Fig. 5. Mobile phase: acetonitrile–triethylammonium phosphate buffer (pH 3.0; 0.05 M) (48:52, v/v) at a flow rate of 1.3 ml min^{-1}. Detection: as in Fig. 5.

gave worse results. Under the chosen conditions (110°C for 50 min and 0.03 ml 60 mM potassium carbonate) the yield of the ether increases to reach a plateau at a reagent to chlorophenol molar ratio of about 100 and further reagent excess does not interfere (Fig. 4). The described reaction conditions were also found to be suitable for the derivatization of chloroxylenol.

The effect of the chloro substituent position in the aromatic ring on the reaction course was also evaluated. As expected, chlorophenols with the chloro substituent in the *meta* position showed weak reactivity.

3.2. Chromatography

Chromatographic separations were carried out under isocratic conditions at 35°C on a reversed phase column (RP-8). The effects of composition and pH of the mobile phase on the resolution and fluorescence intensity of the ethers were investigated. A phosphate buffer (pH 3.0) solution was found to be suitable for fluorescence detection ($\lambda_{exc} = 360$ nm; $\lambda_{em} = 500$ nm). Thus, for the chloroxylenol determination mixture A–B (52:48, v/v) was used, where A was acetonitrile–tetrahydrofuran (95:5, v/v) and B was triethylammonium phosphate buffer (pH 3.0; 0.05 M), at a flow rate of 1.3 ml min^{-1}, while for other chlorophenol analyses a binary mixture of acetonitrile–triethylammonium phosphate buffer (pH 3.0; 0.05 M) (52:48 or 48/52, v/v) at flow rate of 1 or 1.3 ml min^{-1} was chosen. For the chloroxylenol determination the modifier tetrahydrofuran was used to reduce the analysis time without compromising the resolution. Representative HPLC separations of the derivatized chloroxylenol, chlorocresol and related compounds are reported in Figs. 5–7, respectively. As can be seen, the reagent (I) and its degradation products, developed under the reaction conditions, did not interfere with the analysis because they were eluted close the solvent front and before the derivatized phenols. Excitation at 360 nm (second absorption maximum) was chosen

Table 1
Data for calibration graphs

Compound	IS	Slope	y-Intercept	Correlation coefficient
Chlorocresol	4-Chlorophenol	0.0076	0.0289	0.9991
Chloroxylenol	Chlorocresol	0.0049	0.0115	0.9993

$n = 6$, Obtained by the HPLC method for chlorocresol (26.10–130.50 nmol ml^{-1}) and chloroxylenol (48.40–242.00 nmol ml^{-1}) with internal standard (4-chlorophenol, 73.88 nmol ml^{-1} and chlorocresol, 78.03 nmol ml^{-1}) derivatized with 2-chloro-6,7-dimethoxy-3-quinolinecarboxaldehyde.

in order to avoid interference from low wavelengh absorbing analytes.

The detection limit (signal to noise ratio = 3) was about 1 pmol of the injected chlorophenol ethers. However, preliminary investigations carried out, with a view to wider applications of the proposed reagent, showed sensitivity decreased with increasing numbers of chloro substituents on the phenyl ring.

3.3. Analysis of chlorocresol and chloroxylenol in pharmaceutical formulations

Four commercial pharmaceutical ointments or creams, used for skin infections and containing a small percentage of chlorocresol (0.1%, w/w) and chloroxylenol (0.4%, w/w) were analysed by the proposed HPLC method, based on precolumn derivatization with the reagent (I) and fluorimetric detection.

Table 2
Results for the HPLC determination of chlorocresol and chloroxylenol in commercial pharmaceutical formulations

Drug	Formulation	Found*	R.S.D. (%)
Chlorocresol	Ointment[a]	98.99	0.54
	Cream[b]	98.93	1.2
	Ointment[c]	97.60	0.75
Chloroxylenol	Cream[d]	98.17	1.0

Other ingredients: [a] betamethasone valerate, polyoxyethylene cetylstearic ether, cetylstearyl alcohol, white vaseline, liquid paraffin, sodium phosphate monobasic, sodium hydroxide; [b] gentamicin phosphate, polyethylene glycol monocetylether, cetylstearyl alcohol, white vaseline, liquid paraffin, sodium phosphate monobasic; [c] clobetasone butyrate, glycerylmonostearate, cetylstearyl alcohol, white wax, autoemulsifying glyceryl monostearate, dimethicone, glycerol, sodium citrate, citric acid; [d] benzyl alcohol, benzocaine, stearic acid, cetyl alcohol, glycerol, white mineral oil, isopropyl myristate palmitate, polysorbate, cacoa butter, triethanolamine, carbopol, sorbitan tristearate, methylparaben, propylparaben, eugenol, butylhydroxyanisole.
* Mean of five determinations and expressed as a percentage of the claimed content.

Under the described chromatographic conditions a linear relationship between peak-height ratio (analyte to IS) and analyte concentration (nmol ml^{-1}) were found for each drug (Table 1).

The precision of the methods was satisfactory as indicated by the R.S.D. obtained from replicate ($n = 8$) analyses (derivatization and HPLC separation) of a single standard solution of chlorocresol (75 nmol ml^{-1}; R.S.D. 0.5%) and chloroxylenol (145 nmol ml^{-1}; R.S.D. 1.05%).

The commercial pharmaceuticals were of complex composition (Table 2). DMSO, chosen as the solvent for the derivatization reaction, proved to be also convenient for sample dissolution providing a clear analytical solution suitable for the subsequent derivatization reaction.

Thus, commercial formulations containing chlorocresol (cream and ointments) and chloroxylenol (cream) were easily subjected to the derivatization and HPLC analysis with fluorimetric detection ($\lambda_{exc} = 360$; $\lambda_{em} = 500$ nm). The results obtained (Table 2) were found to be in agreement with the claimed content for the preservatives. The other ingredients of the formulations did not interfere with the analysis. The accuracy of the method was verified by analysing commercial samples spiked with known amounts preservatives (20% of the claimed content): essentially quantitative recoveries were obtained for both chlorocresol (98.2–99.7%) and chloroxylenol (98.8%).

4. Conclusions

2-chloro-6,7-dimethoxy-3-quinolinecarboxaldehyde has been found to be a useful fluorogenic reagent suitable for the derivatization of *o*- and *p*-chlorophenols. The reaction provides highly fluorescent ethers that can be separated by reversed phase HPLC and fluorimetrically detected. The reagent degradation products did not give significant interfering peaks under the chromatographic conditions used. The proposed HPLC method proved to be suitable for the determination of chlorocresol and chloroxylenol in commercial pharmaceutical formulations of complex composition without extraction procedures. The strong conditions required for the determination are compensated by its high selectivity, allowing direct sample analysis, and by the opportunity of obtaining simplified chromatograms with high sensitivity.

Acknowledgements

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References

[1] C. Burgess, J. Chromatogr. 149 (1978) 233–240.
[2] L. Campanella, M.P. Sammartino, R. Sbrilli, M. Tomassetti, J. Pharm. Biomed. Anal. 10 (1992) 751–755.
[3] M.S. Bloomfield, K.A. Prebble, J. Pharm. Biomed. Anal. 10 (1992) 775–778.
[4] L. Gagliardi, A. Amato, A. Basili, G. Cavazzutti, E. Gattavecchia, D. Tonelli, J. Chromatogr. 325 (1985) 353–358.
[5] N. De Kruijf, A. Schouten, M.A.H. Rijk, L.A. Pranoto-Soetardhi, J. Chromatogr. 469 (1989) 317–328.
[6] L. Gagliardi, G. Cavazzutti, L. Turchetto, F. Manna, D. Tonelli, J. Chromatogr. 508 (1990) 252–258.
[7] C. Di Nunzio, G. Parisi, P. Santoro, P.A. Ricci, J. Chromatogr. 392 (1987) 454–459.
[8] B. Del Castillo, J. Alvarez-Buila, D.A. Lerner, Fluorogenic reagents and fluorescent probes, in: W.R.G. Baeyens, D. De Kenkeleipe, K. Korkidis (Eds.), Luminescence Techniques in Chemical and Biomedical Analysis, Practical Spectroscopy Series, M. Dekker, Inc., New York, vol. 12, 1991, pp. 73–139.
[9] K. Blau and J.M. Halket, Handbook of Derivatives for Chromatography, 2nd ed, Wiley, New York, 1993.
[10] O. Meth-Cohn, B. Narine, Tetrahedron Lett. 23 (1978) 2045–2048.

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