FIA-SPECTROPHOTOMETRIC DETERMINATION OF RESORCINOL IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A flow injection analysis-spectrophotometric method has been developed for the determination of resorcinol in pharmaceutical formulations. The method involves a previous clean-up of samples by means of a solvent extraction with $CHCl_3$, the consecutive extraction of resorcinol with water and the reaction with p-aminophenol in the presence of KIO_4, being resorcinol determined spectrophotometrically at 540 nm. The method has a limit of detection of 6.6 ng ml^{-1} and provides accurate results in the analysis of real samples.

INTRODUCTION

Resorcinol (1,3-benzene diol) is widely used in antiacne pharmaceutical formulations, especially as an ingredient of Anil, Acnomel and Eskamel formulations, due to its keratolytic, and antiseptic properties[1-2].

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Many methods have been developed for the determination of resorcinol in drugs and pharmaceutical formulations. Gas chromatography (GC) has been employed for the analysis of thermally stable drugs[3]. High performance liquid chromatography (HPLC) has been used in the field of drugs analysis and clinical samples, and hence, resorcinol was determined in cream hair dye, using UV-detection at 280 nm[4], in human serum and urine, by amperometric detection using a vitreous-carbon electrode[5], in tobacco by using fluorometric detection[6] and in pharmaceutical formulations with UV-detection at 254 nm[7].

Spectrophotometric methods have been also employed for the determination of resorcinol by derivatization techniques. For this purpose, many reagents have been recommended, such as cathecol, HIO_4 and sodium nitrite, which react with resorcinol to form coloured species which absorb at 558, 380 and 480 nm respectively[8-10].

The recent developments of the flow analysis techniques (FIA)[11-12] offer a lot of possibilities for the automatization of the spectrophotometric determinations providing an appreciable saving in terms of reagents, time and consumable, and thus, several FIA-spectrophotometric procedures have been proposed for the determination of resorcinol in surface waters.

A FIA procedure has been developed for the on-line condensation of resorcinol with 4-aminoantipyrine (4AAP) and measurement of the oxidizing reaction product at 470 nm[13]. On the other hand, 3-methyl-2-benzothiozoline hydrazone (MBTH) has been employed as a good derivatization reagent for the resorcinol determination in order to form a coloured species which absorbs at 510 nm providing better selectivity and sensitivity than that found on using 4-AAP[14].

Based on our previous experience on the use of p-aminophenol (PAP) as a derivatization reagent for the determination of phenolic compounds[15-19] and specially for the FIA-spectrophotometric determination of resorcinol in water samples[20], we have tried to develop an adequate methodology for the analysis of real pharmaceutical formulations which could be applied for the direct quality control of resorcinol in this field.

EXPERIMENTAL SECTION

A Hewlett Packard 8452A diode array spectrophotometer with a HP 89530A MS-DOS UV/visible software, with a response time of 0.1 s, and a flow cell with 50 μl internal volume and 1 cm pathlength was used for the spectrophotometric measurements.

A three-channels manifold (Fig.1) was used for the flow injection spectrophotometric determination of resorcinol with PAP. The manifold includes a Gilson P2 minipulse peristaltic pump to transport the carrier streams, and a Rheodyne type 50 rotary injection valve used for the introduction of standards and samples.

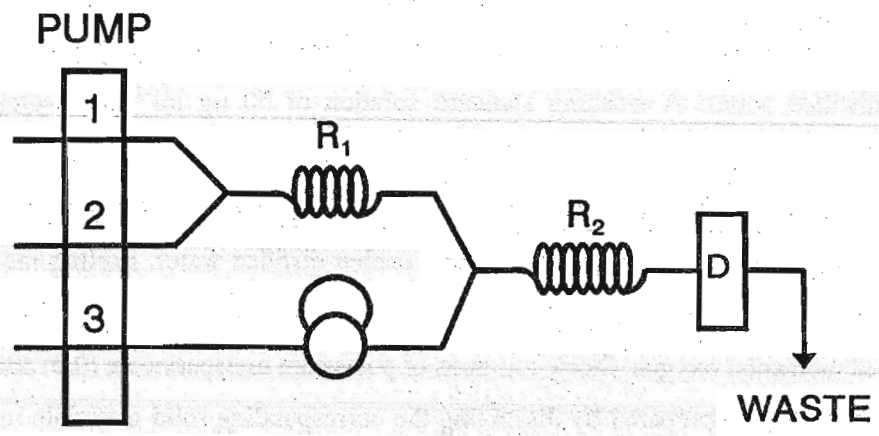


Figure 1: Manifold used for the FIA-spectrophotometric determination of resorcinol with PAP. R_1 and R_2 reaction coil. D, detector.

1) 50 μg ml^{-1} PAP; 2) 0.0002 M KIO_4; 3) 0.006 M NaOH; Flow rate 3.5 ml min^{-1} in each channel; Length of the reaction coils R_1 = 45 cm and R_2 = 3 m; Injection volume = 500 μl.

The connection between any two channels was made by Y-shaped merging zones in order to provide a good mixing between reagents.

Flexible vinyl tubes of 1.52 mm internal diameter were used for the peristaltic pump in order to obtain carrier flow rates up to 3.5 ml min^{-1} in each channel. The reaction coils R_1 and R_2 were made of Teflon, with an internal diameter of 0.8 mm.

An ultrasonic water bath (Selecta Co., Spain) was employed to homogenize the sample solutions or dispersions before their extraction.

Reagent solutions and samples

All reagents employed were of analytical grade: Resorcinol was supplied by Aldrich (Germany), p-aminophenol (PAP) by Fluka (Switzerland), potassium metaperiodate and sodium hydroxide were obtained from Probus (Spain).

Standard stock solution of 100 μg ml^{-1} of resorcinol was prepared using high purity distilled water. A working standard solution of 50 μg ml^{-1} of resorcinol was prepared by diluting the stock one with distilled water.

Standard solution of PAP of 50 μg ml^{-1} was daily prepared by dissolving 0.0125 g of the solid product in 250 ml of boiled and cooled distilled water. Boiling the distilled water for 10 min is very important in order to avoid the oxidation of PAP by the dissolved molecular oxygen. Stock solutions of potassium metaperiodate (0.01286 M) and 1 M of NaOH were prepared by dissolving the corresponding solid materials in distilled water.

The following pharmaceutical formulation samples were bought in spanish pharmacies: Acnosan® a pale yellow liquid solution which is used as an antiseptic, disinfectant, antitallow, antipuriginous, antimicotic and keratolytic agent. Acnisdin®, a brown suspension used as antiseptic, Acnomel®, a brown ointment used for the external applications as an anti acne agent and Anti Acne Leo®, a brown ointment used for the treatment of youth acne, and blackheads. Table I indicates the composition of the four formulations analyzed.

Table I: Composition of the pharmaceutical products analyzed.

Formulation	Composition	Content
Acnisdin®	Calcium dobesilate	2.500 g
	Resorcinol	2.000 g
	Sublimated sulphur	2.000 g
	Excipient (s.q.f)	1000 ml
Acnomel®	Resorcinol	0.020 g
	Sulphur	0.080 g
	Alcohol	0.110 g
Acnosan®	Campher	12.80 g
	Undecylenic acid	8.750 g
	Boric acid	6.450 g
	Resorcinol	1.490 g
	Salicylic acid	1.2000 g
	Zinc sulphate	0.130 g
	Cuprous sulphate	0.130 g
	Aromatic vehicule (s.q.f)	1000 ml
Anti Acne Leo®	2-4-4'-tricloro-2'-hydroxydiphenyl ether	0.250 g
	Resorcinol	2.000 g
	Sulphur	8.000 g
	Alcohol	11.000 g
	Excipient	-

s.q.f. : sufficient quantity for...

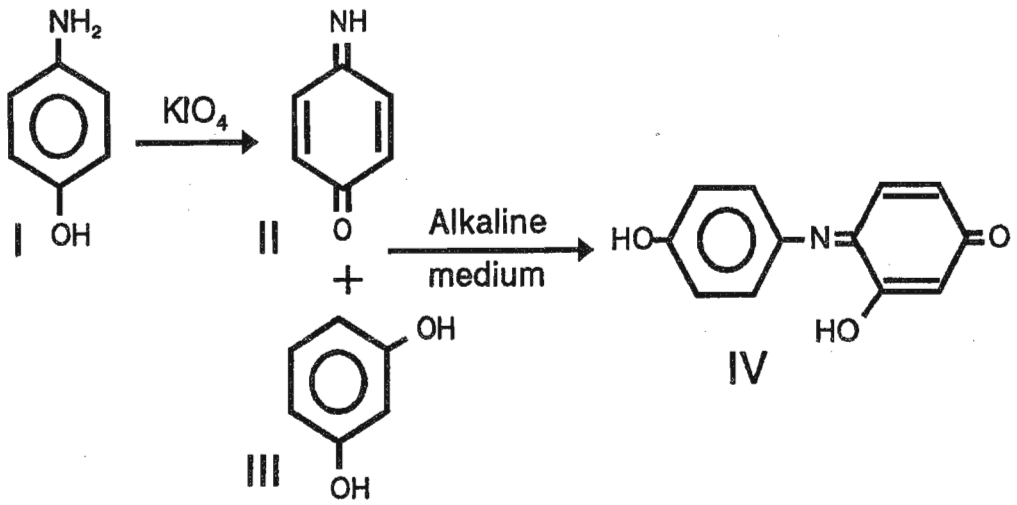
General procedure

Adequate quantities of the aforementioned pharmaceutical formulation samples are accurately pipetted or weighed, diluted with distilled water and shaken for 5 min in an ultrasonic water bath. 50 ml of the above solutions or dispersions are taken in a separating funnel of 100 ml and 20 ml of chloroform added. Funnels are shaken for five minutes and the aqueous phase separated and filtered through a Whatmann filter paper No-42. From the filtrate, low concentrated solutions are prepared in 0.006 M NaOH and 500 μl volumes injected in the manifold using 50 μg ml^{-1} of PAP, 0.0002 M KIO_4 and 0.006 M NaOH as carrier solutions with flow rate values of 3.5 ml min^{-1} in each channel. The FIA-spectrophotometric measurements were carried out at 540 nm against a standard calibration graph of resorcinol.

RESULTS AND DISCUSSION

Spectrophotometric determination of resorcinol with PAP

Resorcinol (1,3-dihydroxybenzene)(III) reacts very fastly with the benzoquinoneimine (II) obtained from the oxidation process of PAP (I) in an alkaline medium to produce a coloured species of indodye (IV) which presents a maximum absorption intensity at 540 nm, according to the following reaction scheme:



In this reaction, resorcinol (III) behaves as an electron donor, due to the presence of two hydroxyl groups, while benzoquinoneimine acts as an acceptor.

The effect of different parameters on the above reaction have been studied elsewhere[19] and experimental conditions for FIA determination are summarized in figure 1.

A typical calibration line obtained in the best experimental conditions corresponds to the expression A = 0.004 + 0.0274 C, being A the peak height absorbance values of the corresponding FIA recording, in absorbance units and C the concentration of resorcinol in $\mu g\ ml^{-1}$. The regression coefficient obtained was 0.9991 for a concentration range between 0 and 6 $\mu g\ ml^{-1}$. The limit of detection corresponds to 6.6 $ng\ ml^{-1}$ of resorcinol and the repeatability of the procedure can be estimated by a typical relative standard deviation of 5 independent analysis of a sample containing 4 $\mu g\ ml^{-1}$ of resorcinol, which corresponds to 0.3 %.

Analysis of pharmaceutical formulations

Extraction method

In order to develop an adequate methodology for the determination of resorcinol in pharmaceutical formulations, it has been developed an extraction procedure which makes possible to avoid interferences in the reaction between resorcinol and PAP due to the presence of other different active principles and excipient. Resorcinol is soluble in water and its solubility corresponds to 1 mg in less than 1 ml of water. This fact has been employed to extract and separate resorcinol from pharmaceutical formulations by using high purity distilled water.

Solvent extraction with chloroform has been used as a clean-up preparatory step in order to remove non polar compounds which provide high background values and excess errors in the determination of resorcinol with PAP or which can cause troubles in

the water extraction of resorcinol from samples. The effect of chloroform on the extraction of resorcinol was evaluated by the comparison of two calibration graphs obtained from aqueous solutions of resorcinol (from 0.5 to 2.5 $\mu g\ ml^{-1}$), both directly prepared and after a previous treatment with chloroform. The spectrophotometric measurements, carried out in batch at 274 nm, for both series of aqueous solutions of resorcinol provided the following two regression equations:

$A_1 = -0.003 + 0.0164\ C$ (C in $\mu g\ ml^{-1}$) with r= 0.9996

$A_2 = 0.001 + 0.0164\ C$ (C in $\mu g\ ml^{-1}$) with r= 0.9997

where A_1 and A_2 correspond to data found without and after a chloroform treatment. These data indicate that the use of a previous extraction with chloroform has no effect on the sensitivity of the measurements nor on the solubility of resorcinol, and it means that chloroform is a suitable solvent to extract other soluble components, which could accompany resorcinol in pharmaceutical formulations, without causing loses in the recovery of the active agent. Several parameters, such as the effect of chloroform and water volume, and the effect of the sample mass on the extraction of resorcinol, using the Anti Acne Leo® ointment as a test system, were subjected to an intensive study.

Effect of the chloroform volume

0.4133 g of the Anti Acne Leo® ointment were dispersed in 250 ml of distilled water, 50 ml of a solution were extracted with different volumes of chloroform (from 5 to 25 ml). After the separation of chloroform, the resorcinol aqueous solutions were analyzed by the general procedure previously indicated. Results obtained are shown in table II, which indicate that the recovery percentage of resorcinol in all cases is higher than 95 %. As the quantity of chloroform increases, 100 % recovery is achieved with 20 and 25 ml of chloroform, thus 20 ml of chloroform was chosen as the most appropriate volume.

Table II: Effect of chloroform volume on the recovery of resorcinol in the analysis of an ointment sample.

Chloroform (ml)	Recovery %
5	96
10	97
15	99
20	100
25	100

In order to demonstrate the suitability of the use of 20 ml of chloroform to extract excipient additional confirmation, experiments were carried out by preparing five independent real samples which contain 2.5 $\mu g\ ml^{-1}$ of resorcinol extracted with 50 ml of water, the samples provided an average value of $2.5 \pm 0.03\ \mu g\ ml^{-1}$ of resorcinol which corresponds to a recovery percentage of 99 ± 1 %.

Effect of water volume

In order to study the effect of the water volume on the extraction of resorcinol from ointment samples, an accurate weight of Anti Acne Leo® was dispersed in water and low concentrated working dispersions prepared in different water volumes (from 20 to 60 ml) after treatment with 20 ml of chloroform.

The two phases were shaken for 5 min, after that the chloroform discharged and the aqueous phase was filtered, alkalinized and injected in the FIA manifold in order to

be analyzed against a series of aqueous resorcinol standards.

The values indicated in table III evidence that the use of a water volume higher than 40 ml provides a quantitative recovery of resorcinol from complex matrices treated previously with 20 ml of chloroform, thus it confirms that the developed methodology could be applied for the analysis of real samples.

Table III: Effect of water volume on the recovery of resorcinol in the analysis of an ointment sample.

H_2O (ml)	Recovery %
20	95
30	96
40	100
50	100
60	100

Effect of sample mass

The effect of different sample weights on the proposed extraction method indicates that it is not a very important factor to be taken into consideration (see table IV as an example), and that quantitative results can be found by using a sufficient amount of sample.

Table IV: Effect of the sample weight on the extraction of resorcinol with water from an ointment sample.

Weight of ointment (mg)	Indicated value $\mu g\ ml^{-1}$	Obtained value $\mu g\ ml^{-1}$	Recovery %
11.1	2.5	2.48	99.1
22.0	2.5	2.50	100
34.7	2.5	2.50	100
41.9	2.5	2.50	100

Analysis of real samples

Four pharmaceutical products of different types, and containing different components other than resorcinol, were analyzed by the recommended procedure and results found by different independent analysis, carried out with different sample quantities, are summarized in table V in which it can be seen that accurate and precise values were found in all cases.

CONCLUSION

The FIA-spectrophotometric method proposed for the determination of resorcinol in pharmaceutical formulations after a previous clean-up with chloroform and extraction of resorcinol with water is accurate, very rapid, simple, highly sensitive and economic and it provides a sample frequency of more than 300 injections per hour, thus the method can be useful for the routine analysis of pharmaceuticals.

The previous extraction of liposoluble components with chloroform is necessary in order to avoid high background values and to obtain good recovery values for resorcinol.

However excess errors obtained when samples are analyzed without a previous clean-up are not due to interferences of the reaction under study but to non specific matrix effects.

Table V: Determination of resorcinol in pharmaceutical formulations.

Pharmaceutical formulations	Real concentrations μgml^{-1}	Found μgml^{-1}	Relative standard deviation %*
Anti Acne Leo®	0.53	0.53	1.4
	1.10	1.10	4.8
	1.58	1.61	3.6
	2.10	2.09	1
Acnosan®	0.62	0.60	0.8
	1.24	1.21	2
	1.86	1.78	0.07
	2.48	2.35	5
Acnomel®	0.39	0.38	0.5
	0.77	0.76	0.7
	1.16	1.09	1.1
	1.54	1.51	0.2
Acnisdin®	0.449	0.443	2.7

* The relative standard deviation values were determined from five independent analysis of each sample.

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REFERENCES

(1) A.C. Moffat, J.V. Jackson, M.S. Moss, B. Widdop and E.S. Greenfield; Clarke's Isolation and Identification of drugs, 2nd.ed, The pharmaceutical press (1986).

(2) M.S. Roberts, R.A. Anderson and J. Swarbrik, J.Pharm. Pharmacol., 29, 677 (1977).

(3) R.E. Ardrey and A.C. Moffat; J.Chromatogr., 220, 195 (1981).

(4) D. Ehlers, Lebensmittelchem-Gerichtl-Chem., 30, 75, (1983).

(5) F. Palmisano, A. Guerrieri and P.G. Zambonin, Ann. Chim. (Rome)., 76, 419, (1986).

(6) H.C. Risner and S.L.Cash, J.Chromatogr.Sci., 28 , 239, (1990).

(7) A.K. Sen, Indian-Drugs., 27, 316, (1990).

(8) R.T. Sane, B.D. Baname and J.G. Mhalas, J.Indian Chem. Soc., 60, 381, (1983).

(9) J.P. Rawat and K.P.S. Muktawat, MicroChem.J., 30, 289, (1984).

(10) M.S. Hasan, B.F. Salem and N. Abd-El-Salem, Anal.Lett., 20, 677, (1987).

(11) J. Ruzicka and E.H. Hansen, (eds), Flow Injection Analysis, Willy, New York, 2nd.ed., (1988).

(12) M. Valcarcel and M.D. Luque de Castro, Flow Injection Analysis, Principles and Application, Ellis Horwood, Chichester, (1987).

(13) J. Moller and M. Martin, Fresenius Z. Anal. Chem., 329, 728, (1988).

(14) W. Frenzel and J. Oleksy-Frenzel, Anal.Chim.Acta., 2661, 253, (1992).

(15) K.D. Khalaf, J. Sancenon and M. de la Guardia, Anal Chim. Acta., 266, 119, (1992).

(16) K.D. Khalaf, J.Sancenon and M. de la Guardia, Fresenius J.Anal.Chem., 347, 52, (1993).

(17) K.D. Khalaf, J. Sancenon and M. de la Guardia, Talanta., 40, 1173, (1993).

(18) K.D. Khalaf, A. Morales-Rubio and M. de la Guardia, Anal.Chim. Acta., 280, 231, (1993).

(19) K.D. Khalaf, B.A. Hasan, A. Morales-Rubio and M. de la Guardia, Microchim. Acta., 112, 99 (1993).

(20) K.D. Khalaf, B.A. Hasan, A. Morales-Rubio and M. de la Guardia, Talanta., 41, 547, (1994).

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